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Review

Spermidine as a target for cancer therapy

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ABSTRACT

Spermidine, as a natural component from polyamine members, is originally isolated from semen and also existed in many natural plants, and can be responsible for cell growth and development in eukaryotes. The supplementation of spermidine can extend health and lifespan across species. Although the elevated levels of polyamines and the regulation of rate-limiting enzymes for polyamine metabolism have been identified as the biomarkers in many cancers, recent epidemiological data support that an increased uptake of spermidine as a caloric restriction mimic can reduce overall mortality associated with cancers. The possible mechanisms between spermidine and cancer development may be related to the precise regulation of polyamine metabolism, anticancer immunosurveillance, autophagy, and apoptosis. Increased intake of polyamine seems to suppress tumorigenesis, but appears to accelerate the growth of established tumors. Based on these observations and the absolute requirement for polyamines in tumor growth, spermidine could be a rational target for chemoprevention and clinical therapeutics of cancers.

1. Introduction

Cancer is a global health problem and one of leading causes of mortality worldwide in the last decades, and the primary prevention of cancers is an area of great interest from scientific, economic, and political levels [1,2]. Numerous risk factors associated with the progression of cancers including environment, genetic hallmarks and lifestyle factors such as diets, smoking, overweight and physical inactivity have been confirmed [3,4]. Due to the genomic instability and phenotypic variation during tumor progression, a potential therapeutic demand is to understand the underlying mechanisms and to drive the combinatorial interventions including drugs, diets and exercise for providing potentially great advantages with intrinsic multi-target effects during the prevention and treatments of cancers.

Spermidine as one of the polyamine members is a trivalent cationic compound found in eukaryotic cells, particularly abundant in sperm. By interacting with nucleic acids, proteins, ATP and other polyanions through electrostatic binding, spermidine is indispensable in cell division and proliferation through maintaining DNA genomic homeostasis, regulating gene transcription and translation, and modulating autophagy, apoptosis, oxidative stress, angiogenesis and cell-to-cell communication [5]. Spermidine has been proven to be a precursor for the essential enzymatic modification of eIF5A, and is required for cell proliferation and viability [6]. Importantly, there is growing consensus that spermidine can induce autophagic flux through reducing acetylation level in cells, thereby displaying pleiotropic effects including

Abbreviations: Ac-PUT, *N*-acetylputrescine; Ac-SPD, *N*-acetylspermidine; Ac-SPM, *N*-acetylspermine; AD, Alzheimer's disease; ADC, arginine decarboxylase; AdoMetDC, adenosylmethionine decarboxylase; APAO, *N1*-acetylpolyamine oxidase; Arg1, arginase 1; AZIN, ornithine decarboxylase antizyme inhibitor; CoA, acetyl coenzyme A; CQ, chloroquine; CRM, caloric restriction mimic; CVD, cardiovascular disease; DAc-SPD, *N1, N12*-diacetylspermidine; DAc-SPM, *N1, N12*-diacetylspermine; dcSAM, decarboxylated S-adenosylmethionine; DC, monocyte-derived macrophages and dendritic cells; DENSPM, *N1, N11*-diethylnorspermine; DFMO, difluoromethylornithine; DMH, dimethylhydrazine; eIF5A, eukaryotic translation factor 5A; HCQ, hydroxyl chloroquine; HDAC, histone deacetylase; IDO1, indoleamine2,3-dioxygenase 1; IL, interleukin; MDSC, myeloid-derived suppressor cell; MGBG, methylglyoxal-bis(guanylhydrazone); MTA, methylthioadenosine; ODC, ornithine decarboxylase; PA, polyamine; PBT, polyamine blockade therapy; PPARγ, proliferator-activated receptor-γ; PPG, prodrug polyamine analog gefitinib; PTI, polyamine transport inhibitor; PUT, putrescine; SAM, S-adenosylmethionine; SAM486A, an inhibitor of S-adenosyl-methionine-decarboxylase, CGP48664; SMO, spermine oxidase; SMS, spermine synthase; SPD, spermidine; SPM, spermine; SRS, spermidine synthase; SSAT, spermidine/spermine *N1*-acetyltransferase; STAT, signal transducers and activators of transcription; TAM, tumor-associated macrophage; TNF-α, tumor necrosis factor-alpha

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promoting lipid metabolism, accelerating anti-inflammation, improving antioxidant activity, and enhancing mitochondrial metabolism and respiration [7–9]. Autophagy plays a crucial role in cell differentiation and tissue remodeling, ensures cellular homeostasis and proteostasis, and acts as a cell housekeeper by preventing the accumulation of damaged or toxic proteins and organelles, thereby enhancing the resistance to cellular stress during the progression of aging and diseases [10–12]. Polyamine synthesis is down-regulated in the senescent status of many tissues [13]. Consistently, the appropriate level of spermidine *in vivo* has been assumed to be beneficial to longevity in an autophagy-dependent manner. During aging process, the content of polyamines in whole blood of elderly population presents a gradually decreasing trend as the extension of age [14]. Moreover, new evidence shows that spermidine can protect from pathological events including two major death causes: cardiovascular disease (CVD) and cancer [15], and other aging-related diseases such as cognitive impairment during Alzheimer's disease (AD) and Parkinson's disease (PD) [16,17].

With regard to cancer, the dys-regulation of polyamine metabolism is a defined signature of many types of tumors [18]. As a caloric restriction mimetic and autophagy inducer, spermidine can reduce the growth of transplantable tumors, stimulate immune surveillance in combination with chemotherapy, and suppress tumorigenesis induced by chemical insults in mice [19]. Dietary polyamine supplementation is correlated with lower cardiovascular disease and cancer-related mortality in human. A recent epidemiological study has documented a potential association between dietary spermidine intake and prolonged survival in human, suggesting that individuals supplied with long-term spermidine-rich diets are characterized by lower overall mortality including CVD and cancers [15]. Moreover, a protective effect of exogenous polyamines is confirmed in postmenopausal women with colorectal cancer risk-lowering behaviors such as reducing body mass index and increasing fiber intake [20]. Therefore, dietary supplementation with spermidine can be served as a promising prevention strategy in various aging-related health issues. However, based on positive regulation of cell growth and proliferation by polyamines, spermidine at too high level could be detrimental to patients suffering from cancer, aging, innate immunity and cognitive impairment during AD and PD [21]. Spermidine is already present in many foods originating primarily from raw plant and animal tissues in our diets, and the level of spermidine is profoundly affected by its external supply [22]. Thus, it is crucial to quantify spermidine level in dietary sources with safety and tolerability as an adjuvant in current standard management strategies against cancer.

2. The sources of spermidine

Spermidine is involved in many physiological processes of plants and animals. In general, a broad and diverse palette of foods, including plant and animal origin foods, contain spermidine at a high amount. Since spermidine can be adsorbed from dietary sources, it also can be produced by intestinal microorganisms [23]. Spermidine is the polyamine member easily absorbed from human intestine and distributed in the body without degradation through systemic circulation [24]. Thus, spermidine-rich foods can contribute to its increased concentration in multiple systems. High amounts of spermidine are detected in many vegetables and fruits including dried soybean (207 mg/kg), mushrooms (62.4–139.3 mg/kg), green peas (4.5–94.5 mg/kg), lettuce (14.8–104.1 mg/kg), broccoli (24.5–51.8 mg/kg), and mango (30 mg/kg). In grains, Japanese corn, whole grain, brown rice, and millet are also rich in spermidine [21]. Spermidine in plants such as safflower and tea has also been gained the large attention in pharmaceutical industries [25,26]. On the other hand, meat, seafood and dairy foods also have high-level spermidine [27].

Moreover, the products from fermentation processes with polyamine-generating bacteria and fungi used in the food industry can provide the generation of polyamines through microbial strains, which may contribute to the malodorous properties of milk and soybean products such as natto, amaranth grain, durian and a verity of cheeses sometimes [15,22]. Therefore, the circulating level of spermidine is influenced by consuming polyamine-rich foods directly or diets containing spermidine-producing microbiota indirectly.

Given high contents of polyamines in certain types of foods, it is possible that a spermidine-rich diet can delay the development of aging-associated diseases as a promising strategy for promoting healthy aging. Based on theoretical backgrounds of spermidine with the functions of anti-oxidation, anti-inflammation and autophagy induction, spermidine has beneficial effects on human health. However, an excessive polyamine can result in apoptosis and cell transformation, which is sufficient to cause oxidative damage and induce reactive oxygen species (ROS)-like toxic effect associated with many pathological changes including cancers [28,29], suggesting that diets have great impacts on the level of spermidine, and the optimal level of spermidine in human to maintain the optimal status for delaying aging and suppressing cancers still needs to be further investigated.

3. Metabolism of polyamines

Spermidine level *in vivo* is determined by polyamines, predominantly initiated from amino acids such as ornithine, methionine and arginine. There are two principal pathways for the synthesis of spermidine, with the involvement of either direct or indirect aminopropylation of putrescine [30]. Putrescine can be synthesized directly via the decarboxylation of ornithine under the catalysis from the rate-limiting enzyme ornithine decarboxylase (ODC) in mammalian cells (Fig. 1). Although the decarboxylation of arginine under the catalysis from arginine decarboxylase (ADC) seems to be associated with the synthesis of agmatine in bacteria and plants, it is found to encode an ornithine decarboxylase antizyme inhibitor (AZIN), and the purified protein lacks ADC activity [31]. Then, the biosynthesized putrescine can be sequentially converted into spermidine and methylthioadenosine (MTA) by spermidine synthase (SRS) with sequential reactions of aminopropyl group from decarboxylated S-adenosylmethionine (dcSAM), which is converted from S-adenosylmethionine (SAM) by enzymatic role of adenosylmethionine decarboxylase (AdoMetDC) as a

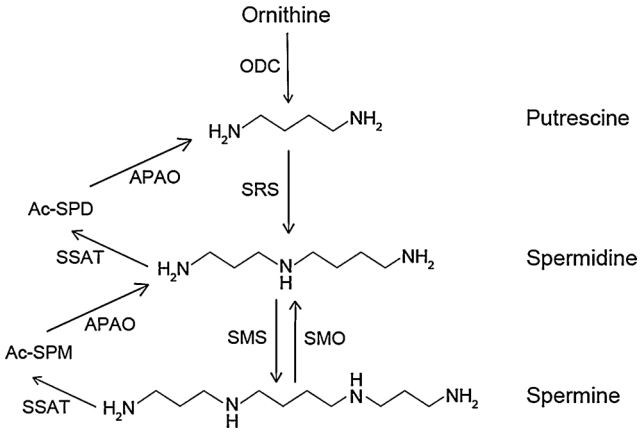

Fig. 1. Polyamine synthesis and catabolism in mammals. Ornithine decarboxylase (ODC) is required for the first step in polyamine synthesis, in which ornithine is decarboxylated to produce putrescine. Decarboxylation of S-adenosylmethionine (SAM) donates its propyl amine moiety for the formation of spermidine and spermine by spermidine synthase (SRM) and spermine synthase (SMS), respectively. The central enzyme in polyamine catabolic pathway is spermidine-spermine-*N*-acetyl transferase (SSAT), which mono-acetylates spermidine and mono-/di-acetylates spermine. These acetylated polyamines are the substrates of N^1-acetylpolyamine oxidase (APAO), which catalyzes their conversion to putrescine. Spermine oxidase (SMO) can be oxidized directly and specifically to produce spermidine by spermine oxidase.

rate-limiting enzyme [32,33]. SMS can produce spermine and an additional MTA molecule from the secondary dcSAM molecule and spermidine. SAM serves as a methyl group donor in many methyltransferase reactions including histone and DNA methylation that are necessary for the epigenetic control of development and aging [34]. Moreover, spermine can be oxidized directly and specifically to produce spermidine by spermine oxidase (SMO), with the production of H_2O_2 and 3-aminopropanal [35]. During polyamine biosynthesis, metabolic factors can affect the bioavailability of arginine, which is important for the production of nitric oxide (NO) as an important signaling molecule in tumorigenesis [36,37].

The catabolic mechanism of spermine and spermidine is a two-step process catalyzing by rate-limiting catabolic enzymes such as spermidine/spermine N^1-acetyltransferase (SSAT) and N^1-acetylpolyamine oxidase (APAO). SSAT plays an important role in polyamine homeostasis with the conversion from spermine and spermidine to monoacetylated metabolites [38]. APAO, a constitutively expressed peroxisomal polyamine oxidase, results in the production of putrescine or spermidine from N^1-acetylspermine and N^1-acetylspermidine, respectively [39] (Fig. 1). In addition to de novo synthesis and degradation, cellular polyamine is also regulated by several polyamine transport proteins classified as ATP-binding cassette transporters and proton potential-dependent carriers [40]. However, these transporters in mammalian cells are not clear.

4. Diagnostic and therapeutic potential for cancers

A series of pathological changes are associated with increased levels of spermidine and other polyamines, thus increasing the possibility of polyamines as the biomarkers in various aspects of human health and diseases including cancers, neurodegenerative diseases, stroke, renal failure [41], heart failure, cardiac infarction and AD [42–44]. A close correlation between polyamine level and tumor progression is well established, and numerous evidence strongly supports the role of polyamines in cancer invasion and metastasis [45]. The polyamines and polyamine metabolites, like spermidine and its acetylated form *N*-acetylspermidine, in either urine or serum, have revealed the potential as the biomarkers for several cancers [46,47]. Since polyamines are upregulated in actively growing cells including cancer cells, the levels of polyamines especially spermidine and the activity of enzymes are often increased in some types of tumors including colorectal carcinoma [48,49]. Polyamines at the higher level caused by enhanced biosynthesis are often be found in different cancer tissues than that in normal tissues, including skin, breast, colon, lung, blood and urine [50–52] (Table 1). Different polyamines and enzyme levels in different cancers are diverse so that different cancers have different metabolisms. Therefore, it may not be optimal to define the complex relationships between cancers and elevated polyamine levels in all type of cancers. Based on higher levels of polyamines in tumors and positive regulation of cell growth and proliferation, the elevated level of spermidine has been recognized as the biomarker for monitoring the growth of tumors [53]. Since the proliferation-enhancing and cytoprotective effects of polyamines on cultured cancer cells or xenografted tumors in immunodeficient mice [54], polyamines may have pro-carcinogenic properties. It is reasonable to use the targeted metabolic pathway of polyamines for the prevention and early diagnosis of cancers, especially in characterizing different types of cancers.

The increased activity of the enzymes involved in polyamine biosynthesis is also observed in tumors, especially ODC [55]. As mentioned above, ODC is overexpressed as a key rate-limiting enzyme in polyamine synthesis pathway and regulated at the transcriptional level by tumor-promoting agents in a variety of cancers [56]. ODC is the target of the oncogene *Myc* as a potential oncogene because it can be overexpressed in transformed mammalian cell lines alone or in combination with other oncogenes [57,58]. Transgenic mouse models overexpressing ODC in skin have demonstrated that ODC can lead to the formation of spontaneous skin tumors, suggesting its possible association with cancerous cells [59]. However, life-long overexpression of ODC gene in transgenic mice does not result in the enhanced spontaneous tumor incidence or neuronal degeneration [60]. Besides, polyamine oxidation through SSAT/APAO pathway is peroxisomal oxidation in the presence of peroxisomal catalase, thus substantially attenuating the production of H_2O_2.

Table 1
Clinical data of polyamines and enzyme levels in tissues from different cancers.

Tissues	Cancer types	Levels	References
Blood	Human hepatic cancer	PUT, SPD↑	[52]
	Breast Cancer	–	[137]
	Lung Cancer	SAM↑	[138]
	Liver Cancer	PUT, SPD, SPM↑	[139]
	Breast, lung and colon cancer	PA, SPM/PUT↑	[140]
Urine	Ovarian Cancer;	DAc-SPM↑	[51]
	Human hepatic cancer	SPD, SPM, Ac-SPD↑	[52]
	Breast Cancer	–	[137]
	Liver Cancer	SPM, Ac-SPM↑	[139]
	Lung Cancer	Ac-SPM, DAc-SPM↑,	[139,141]
	Non-small cell lung cancer	–	[142]
	Colorectal cancer	DAc-SPM↑	[46]
Skin	Squamous-cell carcinoma	PUT↑	[143]
Breast	Invasive Breast Cancer	PUT, SPD, SPM↑	[137]
Colon polyp	Colorectal neoplasia	ODC↑	[144]
Pleural fluid	Lung cancer	SPD↑	[145]
Prostate	Prostate cancer	SSAT, ODC, AdoMetDC↑	[146]
	Prostate cancer, PIN	SMO↑	[147]
Saliva	Pancreatic Cancer	SPM, Ac-SPD, Ac-SPM↑	[47]
	Breast Cancer	Ac-PUT, Ac-SPD, Ac-SPM, DAc-SPD, DAc-SPM↑	[148]
Fingernails	Lung cancer	SPM↑	[149]

Note: PA, polyamines; PUT, putrescine; SPM, spermine; SPD, spermidine; Ac-PUT, *N*-acetylputrescine ; Ac-SPD, *N*-acetylspermidine; Ac-SPM, *N*-acetylspermine ; DAc-SPM, N^1, N^{12}-diacetylspermine; DAc-SPD, N^1, N^{12}-diacetylspermidine; SMO, spermine oxidase; SAM, S-adenosylmethionine ; ODC, ornithine decarboxylase; SSAT, spermidine/spermine *N*-acetyltransferase; AdoMetDC, adenosylmethionine decarboxylase; PIN, prostatic intraepithelial neoplasia.

Because increased polyamine level is usually associated with poor prognosis, and reveals a decrease after tumor eradication and an increase after relapse, the effect and underlying mechanisms of polyamines on the metastasis and invasion of cancer cells have attracted extensive attention for further investigation [61]. In cancer tissues, polyamines reveal an increased level so that the increased uptake of spermine and spermidine may be associated with increased production of proteinases for degrading surrounding tissues [62]. Moreover, polyamines play an important role in inflammation-induced carcinogenesis, with decreased immune functions and enhanced capability of cancer cells for the invasion and metastasis to new tissues as the increased spermidine and spermine levels [61]. These findings implicate another role for polyamines in cell migration or metastasis in cancers.

In recent years, selective inhibitors have been developed for regulating the metabolism of polyamines. Some inhibitors have become as the important tools in elucidating metabolic products of polyamines, but only few of them have been used as the effective inhibitors for controlling the growth of tumors in clinical trials (Table 2). A number of therapeutic practices have been conducted using difluoromethylornithine (DFMO), an inhibitor of ODC, as a chemopreventive agent based on its capability to control the remission of tumors in either animals or human with low toxicity [63,64]. In many tissues such as colonic mucosa, DFMO can suppress putrescine and spermidine,

Table 2
The status of treatments with agents targeting polyamine metabolism in clinical trials.

Tissues	Treatments	Target points	Outcomes	Side effects	References
CNS	DFMO + PCV	Anaplastic glioma	Increased survival	Diarrhea, anemia, ototoxicity	[117]
	DFMO + PCV	Glioblastoma	No additional benefit	Myelosuppression, ototoxicity, fatigue	[150]
	XRT + DFMO	Glioblastoma	No additional benefit	Nausea, diarrhea, myelosuppression, ototoxicity	[151]
	DFMO + MGBG	Primary recurrent malignant brain tumors	Anti-tumor activity mainly for anaplastic glioma	Ototoxicity, tinnitus, diarrhea, when combined with MGBG acute hepatic necrosis	[152]
Lymph nodes	SAM486A	Poor prognosis Non-Hodgkin's lymphoma	Anti-tumor activity	Nausea, vomiting, diarrhea, asthenia, abdominal pain, flushing	[153]
Colorectal	DFMO + sulindac + dietary polyamines	Colorectal adenoma	Reduced metachronous adenoma risk	_	[86]
	DFMO + sulindac	Colorectal adenoma	Suppressed production of rectal mucosal polyamines	_	[87]
	DFMO + sulindac	Colorectal adenoma	Reduced recurrent adenomatous polyps	Cardiovascular amd hearing damage	[154]
	DFMO + celecoxib	Familial adenomatous polyposis	Moderate synergy	Fatigue	[155]
	SAM486A + 5-fluorouracil/leucovorin	Metastatic colorectal cancer	Partial response and stable disease	Neutropenia, hand and foot syndrome, nausea, vomiting, diarrhea, constipation	[156]
Prostate	DFMO	Prostate cancer	Decreased growth of prostate tumor	Hearing damage	[157]
	DFMO + PXM	Prostate cancer	Not assessed	Tinnitus	[158]
	DFMO ± doxorubicin + cyclophosphamide	Prostate cancer	No effect	Ototoxicity	[159]
	DFMO + MGBG	Prostate cancer	No effect	Gastrointenstinal disconforts, fatigue	[160]
Skin	SAM486A	Metastatic melanoma	No effect	Fatigue/lethargy, myalgia and neutropenia	[161]
	DFMO	Nonmelanoma skin cancer	Reduced growth of tumors	Nausea, diarrhea	[162]
	DFMO	Nonmelanoma skin cancer	No significant effect on new NMSCs, significant reduction in basal cell carcinoma	Gastrointestinal disconforts, nausea or diarrhea, hearing damage	[163]
	DFMO + diclofenac	Sun-damaged skin	No benefit	Local cutaneous effects	[164]
	DFMO + triamcinolone	Actinic keratoses	Reduced skin biopsy, nuclear abnormality	_	[165]
Esophagus	DFMO	Esophageal cancer	Reduced cancer risk	Hearing damage	[166]
Non-CNS nerve	DFMO	Neuroblastoma	Increased survival	Grade 2-3 transaminitis	[67]
	DFMO + etoposide	Relapsed/refractory neuroblastoma	Reduced risk of disease progression or death	Hematologic or gastrointestinal issues	[119]
Cervical	DFMO	Cervical intraepithelial neoplasia	No effect	Diarrhea, dizziness, nausea, and headaches	[118]
	DFMO	Cervical intraepithelial neoplasia	Regression of CIN lesions	No major clinical toxicity	[167]
Solid tumor	DENSPM	Advanced malignancies	Moderate response	CNS symptoms	[168]

DENSPM, N^1, N^{11}-diethylnorspermine; SAM486A, an inhibitor of S-adenosyl-methionine-decarboxylase, CGP48664; MGBG, methylglyoxal-bis(guanylhydrazone).

but not spermine [65,66]. DFMO exposure could not suppress the expression of genes involved in cell proliferation, tissue remodeling and tumor invasion *in vitro* in several types of tumors with decreased spermidine and putrescine levels, but can exhibit limited anti-tumor activity *in vivo* and in clinical trials due to compensatory mechanisms upon the occurrence of depleted polyamine pools, suggesting a novel combinatorial therapeutic strategy with polyamine-targeted drugs [67]. Several competitive inhibitors of the enzymes for polyamine biosynthesis, including methylglyoxal bis(guanylhydrazone) (MGBG) and S-adenosyl-methionine-decarboxylase (SAM486A), with the functions of decreasing spermidine and spermine levels and increasing putrescine level, have also been tested *in vitro* and in clinical trials for various types of tumors (Table 2). These inhibitors associated with polyamine metabolism have revealed anti-proliferative effects in cell and animal models, while their successful clinical application in human are focused on glioma, colon cancer and non-melanoma skin cancer [68]. Although it is plausible that the intervention through polyamine signal pathway may not be an effective therapeutic approach as a single agent in other solid tumors [69–72], these inhibitors still can provide a large number of preclinical data along with chemoprevention and additive anti-tumor effect when combined with other agents [68]. In general, these inhibitors are well tolerated in most trials. The treatments at high doses in these trials usually can result in ototoxicity and gastrointestinal toxicity accompanying with hearing loss, diarrhea, fatigue, headache and nausea in a significantly large number of patients (Table 2). The analogues with high similarity in the structures of natural polyamines also have been considered as anti-cancer drugs previously [73,74]. Moreover, recent efforts have been devoted to the development of novel anti-cancer drugs for reducing the viability and migration of cancer cells based on the combinatorial treatment of silencing spermidine synthase (siSPDSYN) and silencing ODC (siODC) associated with spermidine biosynthesis [75].

Although many investigators have reported that spermine and spermidine from diets or other supplements can promote the development and metastasis of tumors, the specific mechanisms have not been defined. The nutritional uptake of spermidine and spermine could be linked with improved cardiovascular health, which does not reveal any significant effect of spermidine on the incidence of cancers [76]. On the other hand, in mice, spermidine can postpone the manifestation of cancers upon oncogenic stimuli [19,77]. Notably, increased polyamine intake seems to suppress 1,2-dimethylhydrazine(DMH)-induced tumorigenesis, but appears to accelerate the growth of the generated tumors [78].

All of these observations suggest that the level of spermidine and its regulation of metabolic pathways can be exploited to predict disease outcome combined with increasingly precise genomic signatures, which will be beneficial to developing more sensitive approaches for cancer diagnosis and treatments based on spermidine as the biomarkers.

5. Molecular mechanisms of spermidine for regulating cancers

Although spermidine seems to be associated with numerous cellular processes, a key criticism of spermidine involving a complex relationship between oncogenes and polyamine metabolism has been observed in cancer therapy. Moreover, spermidine can contribute to the regulation of cancer-related functions, including immune system, autophagy and apoptosis (Fig. 2).

5.1. Polyamine metabolism

The dysfunctional metabolism of polyamines, spermidine and/or spermine, are frequently observed in cancers, indicating that the elevated polyamine level is necessary for the transformation and progression of tumors [79]. The high activity of ODC and AdoMetDC is also determined in rapidly growing tissues and cells, particularly in tumor cells, indicating polyamine biosynthesis as a target for the therapy of cancers. A variety of oncogenes and tumor suppressors can regulate the metabolism of polyamines, thereby resulting in not only increased polyamine biosynthesis, but also increased cellular uptake of polyamines via an up-regulated polyamine transport system. The direct interplay between spermidine and cancer is also regulated by a transcriptional target of *Myc* oncogene. *Myc* as a transcription factor for the development of cancers is critical to neoplastic cells [80,81]. The *Myc* signaling pathway, especially *Myc*-hypusine-polyamine network, appears to be central to the progression of *Myc*-driven cancers by enhancing the expression and activity of ODC, thus providing the stimulation for cells to increase polyamine biosynthesis necessary for the proliferation in multiple types of cancers [82–85]. Spermidine can be utilized as the substrate to complete hypusine synthesis and eIF5A activation, thereby affecting protein synthesis during tumorigenesis in eIF5A-controlled translational mechanisms, and leading to tumor development through direct link between *Myc* and spermidine [86,87].

Except *Myc*, other oncogenes also have been identified to be associated with catabolic enzymes of polyamines. KRAS is implicated in the down-regulation of SSAT via the interference with peroxisome proliferator-activated receptor-γ (PPARγ) transcriptional activation of SAT1, thus allowing transformed cells to maintain the elevated level of spermidine [88]. In a series of cell models with human hepatocellular carcinoma and colon carcinoma, polyamine (spermidine and spermine) depletion through SSAT overexpression can result in the decreased AKT signaling and reduced nuclear β-catenin, thus leading to the decreased cell growth, migration and invasion [89]. Moreover, the tumor-suppressive function of p53 seems to be partially mediated by direct transcriptional activation of SSAT and SSAT-dependent lipid peroxidation in response to ROS-induced cell stress [90].

5.2. Anti-cancer immunosurveillance

The immunosuppression by activating negatively regulatory signal pathways is considered as a major impediment for effective anti-cancer responses [91]. Polyamines have been reported to be involved in the establishment of an immunosuppressive tumor microenvironment by a series of multiple sophisticated mechanisms as the major cause for the failure of most immunotherapeutic regimens, thereby highlighting the regulatory role of polyamines in carcinogenesis-associated immune dysfunction [92,93]. Since the beneficial effects following treatments with ODC inhibitors and polyamine transport inhibitors (PTIs) are reversed in Rag1 mice lacking both T and B-cells and in athymic nude mice lacking T-cells alone, which is consistent with the activation of T-cells after polyamine depletion in tumor models, suggesting an improved activation and/or cytotoxic activity of T cells upon the therapy by polyamine blockers for eliciting anti-tumor immune responses [94]. In the tumor microenvironment, suppressive myeloid cells, including myeloid-derived suppressor cells (MDSCs) and monocyte-derived macrophages and dendritic cells (DCs), are significantly elevated in tumor-bearing animals [95]. It is likely that polyamine blockade therapy (PBT) can activate an anti-tumor immune response via multiple mechanisms affecting the metabolism of both tumor epithelial cells and immunosuppressive tumor-associated cells. The anti-tumor effect of PBT is accompanied by an increase in $CD8^+$ T-cells and a decrease in immunosuppressive tumor-infiltrating cells, and can relieve the systemic inhibition of polyamines in tumor-bearing mice to restore anti-tumor immunity of T-cells [96]. The inhibition of polyamines also can stimulate the production of tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) cytokines by tumor-infiltrating macrophages, suggesting the reprogramming of macrophages into M1 phenotype with augmented tumor-associated antigens [96].

In view of cytokines, tumor-associated macrophages (TAMs) play a critical role in connecting inflammation with cancers, which can be polarized into M1 or M2 macrophages [97]. The activation of M1 macrophages is essential in the host defense response to microbes and tumor cells, while M2 macrophages have a critical role in parasite

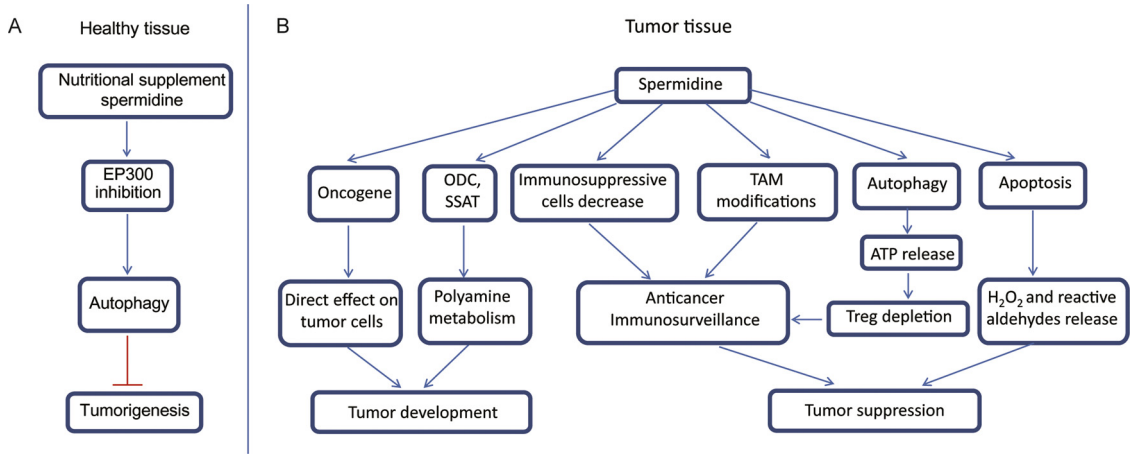

Fig. 2. Cellular and molecular mechanisms of spermidine-mediated diagnostic and therapeutic potentials for cancers. (A) Spermidine may suppress tumorigenesis through the induction of autophagy in healthy tissues. Spermidine competitively inhibits the enzymatic activity of acetyl transferase EP300, thereby resulting in the deacetylation of multiple autophagy-related proteins, and triggering autophagy. (B) In tumor microenvironment, spermidine may exert cell-autonomous effects and polyamine metabolism in cancer cells, and may also influence their communication with immunosurveillance effectors to inhibit immunosuppressive cells and promote M2 polarization. Spermidine also favors the autophagy-dependent release of ATP, which in turn favors immunosurveillance. Apoptosis may be induced by releasing H_2O_2 and reactive aldehydes to suppress the growth of tumors. TAM, tumor-associated macrophages.

defense and external damage [98]. Recent studies have demonstrated that ODC can regulate the activation of M1 macrophages and mucosal inflammation via histone modification, and the myeloid-specific deletion of *Odc* (*Odc*$^{\Delta mye}$) can provoke a marked increase in the response of mouse bone marrow-derived macrophages to M1 stimuli, thus providing new insights into strategies for cancer prevention [99,100]. In addition, M2 macrophages are thought to be associated with arginase 1 (Arg1), which can supply ornithine to ODC and promote tumor progression by inhibiting the activation of cytotoxic T cells. ODC inhibition through DFMO or PTI treatment can inhibit Arg1 activity directly or increase ornithine level through feedback inhibition, thus correspondingly reducing intratumoral suppressive MDSCs [101–103]. In addition, polyamines produced by DC or Arg1$^+$ MDCSs can induce indoleamine-2,3-dioxygenase 1 (IDO1) and turn DCs into an immunosuppressive phenotype cells [104]. In a recent study, the local administration of CRMs including spermidine via aerosol can reduce metastasis in lung, and reveal a significant increase of M1 phenotype such as IL-12 and STAT-1, and a decrease of M2 genes such as IL-10 and STAT-6, to modulate immunosurveillance at tumor sites as a non-invasive therapeutic approach [105]. These observations suggest the regulatory role of polyamine as an immunosuppressive effector by targeting CD8$^+$ T-cells and suppressive myeloid cells to provide a survival mechanism and restore a more conducive tumor microenvironment for evading the immune response in tumors.

5.3. Autophagy

It is noteworthy that autophagy has a context-dependent role in cancers, and the determination of tumor cell fate by autophagy depends on cancer type, stage, and genetic context. By suppressing the initiation of tumor growth at the early stages of cancers, autophagy also leads to the improved survival of tumors as a tumor promoter through providing tumor metabolic substrates to maintain mitochondrial function from metabolic stresses, such as hypoxia and therapeutic stress at the later stages of established cancers [106], thereby providing the possibility to use autophagy inducers or inhibitors as a novel approach for chemoprevention and treatments during tumor progression at different stages [107,108]. Although the association between tumor cell survival and autophagy can be partly explained by the role of autophagy in promoting cell growth, survival and tumorigenesis, called “autophagy addiction” [109–111], phase I/II clinical trials with autophagy inhibition such as chloroquine (CQ) or hydroxyl chloroquine (HCQ) in combination with other drugs or therapeutic strategies have demonstrated the inconsistent evidence in the suppression of tumors [112–115]. In contrast, the studies in mice with autophagy deficiency have demonstrated the increased DNA damage, inflammation and genome instability, which may promote tumorigenesis and tumor progression, suggesting the importance of tumor suppression by autophagy.

By inducing autophagy, caloric restriction mimics (CRMs) such as spermidine, have dual effects on the prevention of tumor initiation and the promotion of tumor progression by assisting in hypoxia-induced switch to anaerobic glycolysis [116]. The treatments by autophagy inducer rapamycin (or everolimus) alone or in combination with chemotherapeutic drugs have revealed autophagic cell death and anti-angiogenesis effect in acute lymphoblastic leukemia, renal cell carcinoma, breast cancer, and pancreatic neuroendocrine tumors [117–119]. Histone deacetylase (HDAC) inhibitors have also revealed autophagy-inducing potential as one of their anti-cancer effects [119]. Similarly, spermidine has been approved as an autophagy inducer and histone acetyl transferase inhibitor by inhibiting EP300, an acetyl transferase for transferring acetyl groups from acetyl coenzyme A (CoA) on lysine residues of proteins, which is well known for its exploitation by cancer cells to achieve and maintain an oncogenic phenotype as a starting material [120–122]. Although it has been common wisdom that the elevated level of polyamine is recognized as cancer biomarker during chemotherapy and appears to accelerate the growth of established tumors, a recent preclinical and clinical observation indicates the treatment effect of spermidine as a CRM on the reduced cancer-related mortality [15]. In human colon cancer cells, spermidine has shown to change the acetylation status of key autophagy-related proteins such as ATG5 and LC3 through increasing the activity of AMP-dependent kinases [123]. Moreover, the possibility to replace chemotherapy by using CRM such as spermidine has also been investigated to reveal the induction of autophagy by improving anti-cancer immunosurveillance during its combinatorial treatment with chemotherapy in cancer-bearing mice [19,124]. This positive effect is achieved by releasing ATP, inducing autophagy, suppressing adenosine production, or accelerating Treg depletion in an autophagy-dependent manner [125]. Beyond these mechanisms, therapeutic strategies involved in the conventional anti-cancer treatments combined with spermidine should be desired at the early stages of cancers by suppressing the initiation of tumor growth.

5.4. Apoptosis

Apoptosis, programmed cell death, represents a major source of cancer cell attrition. Apoptotic program is present as a prominent hallmark of cancers in both extrinsic and intrinsic pathways [126]. The dys-regulated apoptosis with overexpression of anti-apoptotic proteins and/or down-regulation of pro-apoptotic proteins often leads to the accumulation of potentially harmful cells, including cancer cells, and contributes to tumorigenesis and the resistance to anti-cancer treatments [127]. Since the suppression of apoptosis is thought to play a central role in the development and progression of some cancers, targeting critical apoptotic program is an attractive therapeutic way to induce the death of these cells. In contrast to the tumor-suppressor roles of autophagy, stress-activated autophagy may promote the survival of tumor cells, especially when apoptosis is defective [128]. The over-accumulation of polyamines is associated with cell transformation or apoptosis. Recently, increasing evidence suggests that spermidine can suppress the proliferation and promote the death of HeLa cells via autophagic activation *in vitro* [129]. In accordance with these results, synthetic acyl-spermidine derivatives as polyamine analogs have shown pro-apoptotic effects in human breast cancer MCF-7 cell line [130]. In addition, the activation of amine oxidation by diamine oxidase can also cause oxidative stress and apoptosis by the generation of H_2O_2 and reactive aldehydes [131]. Furthermore, maize polyamine oxidase can also induce the apoptosis of LoVo human colon adenocarcinoma cells by catalyzing oxidative deamination of spermine and spermidine to generate H_2O_2 and aldehydes [132]. The tumor repressor p53 with the capability to induce SSAT is involved in ferroptotic cell death, which also provides the insight into the regulation of polyamine catabolism and ferroptosis-mediated tumor suppression [133]. It is noteworthy that major inconsistence is reported in literatures regarding the role of polyamines in apoptosis, due to the complexity of polyamine actions and apoptotic processes.

6. Future remarking

Cancer is a major public health issue worldwide, and numerous studies provide important insights into the underlying mechanisms and therapeutic or diagnostic effects of spermidine on cancers [134–136]. As us understanding of signal pathways affected by polyamines in growth processes of tumors, polyamines and key enzymes associated with polyamine metabolism have applied to clinical practice as the biomarkers and potential targets for the diagnosis and treatments of cancers or the development of anti-cancer drugs. Taking into consideration specific levels of polyamines, it is possible to use the targeted metabolic pathway as the critical biomarker for early diagnosis and treatments, especially in characterizing different types of cancers. Despite the fact that the link between polyamines and cancers has been known for more than several decades, the knowledge of specific mechanisms for polyamine metabolism during carcinogenesis is still less. Moreover, the efficacy of clinical treatment strategies with the aim to regulate metabolic pathways of polyamines is still far away from the success due to the toxicity and side effects, suggesting that future application of structural analogues of natural polyamines in combination with other anti-cancer drugs to deplete polyamine pools should be further explored.

Since spermidine is a natural component originally isolated from semen and existed in many natural plants, it can be supplied as a dietary supplement and clinical trials aiming at increasing its uptake for long-term intervention should be safe, well-tolerated and adopted by general human population. Although spermidine supplementation has various positive effects on health promotion in aging-associated diseases, including CVD, neurodegenerative diseases and cancers, a word of caution must be added due to the double-edged behavior of spermidine or the opposite effects on tumor growth depending on various stages. Based on our knowledge, for an association between diets rich in spermidine and increased survival in human, spermidine supplementation seems to be link with lower cancer-related mortality and the maintenance of autophagic flux required for the suppression of tumorigenesis, so that a low polyamine diet may be also beneficial as cancer chemotherapy in some existed tumors. In summary, the increasing evidence provides the implications for developing spermidine diets or spermidine analogs with a more stably pharmacokinetic profile for the prevention or treatments of cancers.

Authors contributions

J.F. and N.C. participated the design of this study; J.F. and Z.F. completed the literature searching; J.F., Z.F. and N.C. wrote the draft of the manuscript; N.C. conducted the final editing of this manuscript; J.F., Z.F. and N.C. reviewed and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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