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Invited Review

Polyamine metabolism and cancer

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Abstract

Polyamines are aliphatic cations present in all cells. In normal cells, polyamine levels are intricately controlled by biosynthetic and catabolic enzymes. The biosynthetic enzymes are ornithine decarboxylase, S-adenosylmethionine decarboxylase, spermidine synthase, and spermine synthase. The catabolic enzymes include spermidine/spermine acetyltransferase, flavin containing polyamine oxidase, copper containing diamine oxidase, and possibly other amine oxidases. Multiple abnormalities in the control of polyamine metabolism and uptake might be responsible for increased levels of polyamines in cancer cells as compared to that of normal cells. This review is designed to look at the current research in polyamine biosynthesis, catabolism, and transport pathways, enumerate the functions of polyamines, and assess the potential for using polyamine metabolism or function as targets for cancer therapy.

Keywords: polyamines - cancer - ornithine decarboxylase

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Introduction

Polyamines — putrescine ($H_2N(CH_2)_4NH_2$), spermidine ($H_2N(CH_2)_3NH(CH_2)_4NH_2$), and spermine ($H_2N(CH_2)_3HN(CH_2)_4NH(CH_2)_3NH_2$) — are organic cations with multiple functions in cell proliferation and differentiation [1-4]. Polyamine biosynthesis in mammalian cells begins with the production of putrescine by the decarboxylation of the amino acid, ornithine by ornithine decarboxylase (ODC). Subsequent addition of an aminopropyl group to putrescine leads to the synthesis of spermidine and further addition of another aminopropyl group leads to the formation of spermine [1,4]. Aminopropyl group is derived by the decarboxylation of S-adenosylmethionine through the action of S-adenosylmethionine decarboxylase (SAMDC). ODC and SAMDC are rate-limiting enzymes in polyamine biosynthesis. Addition of aminopropyl groups to putrescine and spermidine is catalyzed by the enzymes spermidine synthase and spermine synthase, respectively. Intracellular polyamine levels are also controlled by catabolism, allowing the conversion of spermine back to putrescine [4-6]. For this back-conversion, spermidine and spermine are acetylated by spermidine/spermine acetyltransferase (SSAT). Acetylated spermine is cleaved into spermidine through the action of a flavin adenine dinucleotide (FAD)-dependent polyamine oxidase. Polyamines are also acted upon by diamine oxidases, which are copper-containing amine oxidases [6,7]. In addition to these multi-level control of synthesis and catabolism, polyamine uptake and efflux pathways are responsible for fine-tuning cellular polyamine levels. Linkage between polyamine biosynthesis and uptake exists through ODC antizyme which suppresses polyamine transport and causes the degradation of ODC [7,8]. Fig. 1 shows a schematic representation of the polyamine metabolic pathway.

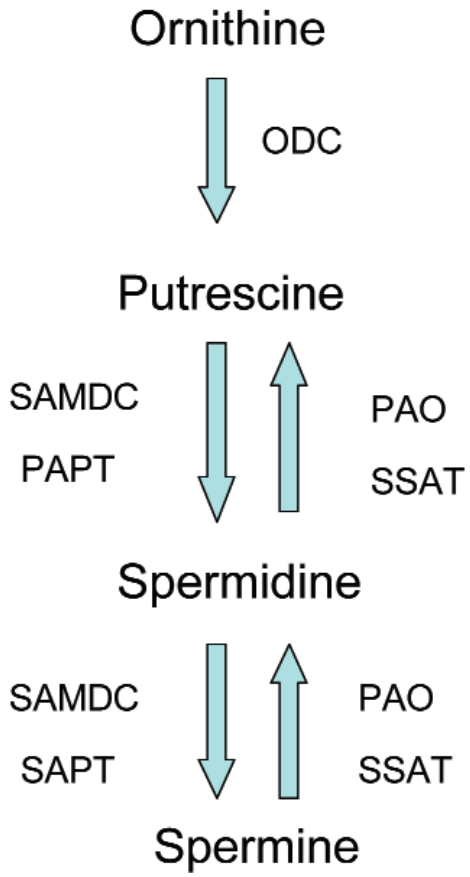

Fig. 1 Pathways of polyamine metabolism. Putrescine is formed by the decarboxylation of ornithine by ornithine decarboxylase (ODC) (E.C.4.1.1.17). Spermidine is formed by the action of spermidine synthase (E.C.2.5.1.16), also known as putrescine aminopropyl transferase (PAPT). Amino propyl group is derived from decarboxylated S-adenosylmethionine, by S-adenosylmethionine decarboxylase (SAMDC) (E.C.4.1.1.50). Spermine is synthesized from spermidine by the addition of an aminopropyl group by spermine synthase (E.C.2.5.1.22), also known as spermidine aminopropyl transferase (SAPT). Polyamine catabolism involves the action of spermidine/spermine acetyl transferase (SSAT) (E.C. 2.3.1.57), and a number of polyamine oxidases (PAO).

Polyamine functions

Clues on polyamine functions can be seen from their chemical structure. These molecules are positively charged at the primary and secondary amino groups at physiological pH. Therefore, electrostatic interactions through the cationic amino groups and hydrophobic interactions through the methylene bridging groups are dominant. Thus, polyamines may act as ligands at multiple sites on DNA, RNA, proteins, phospholipids, and nucleotide triphosphates. While some of these interactions may be purely electrostatic and easily replaced by inorganic cations, others are specific to the length of the aliphatic carbon chain [1-5].

Our studies on polyamines are based on the premise that a large part of the biological function of polyamines is in the regulation of gene expression both by altering DNA structure and by modulating signal transduction pathways. Thus, DNA conformational transitions induced by polyamines can serve as a basis for regulation of gene expression by unravelling DNA-protein interactions and chromatin structure. For example, we and others found that the bending of DNA and the transition of right-handed B-DNA to left-handed Z-DNA are provoked by micromolar concentrations of polyamines [9–11]. It is now well known that DNA curvature alters transcription [12]. DNA sequences that form Z-DNA are frequently found near the transcription start sites [13]. Recent studies suggest that allelic variations in some genes can be attributed to differences in Z-DNA formation by polymorphic dinucleotide repeats [14].

Another facet of polyamine-DNA interactions involves modulation of DNA-protein interactions [4,15,16]. Polyamines enhance the binding of several gene-regulatory proteins to the specific regulatory sequences, called response elements [15,16]. For example, spermine facilitated the binding of estrogen receptor to its response element and nuclear factor κB (NF-κB) to its response element at 100 to 500 μM concentrations [15,17]. In contrast, STAT3 provides an example of a transcription factor binding insensitive to changes in polyamine levels (18), while Oct-1 binding to DNA was inhibited by increased polyamines [16].

Ligand receptor-interactions provide another venue for the action of polyamines. The best characterized system is the effect of polyamines on the function of N-methyl-D-aspartate (NMDA) receptors [19]. NMDA receptors contributes to excitatory synaptic transmission throughout the brain and spinal cord. Polyamines potentiate or inhibit glutamate mediated responses of this channel complex in a concentration-dependent manner [19,20]. In addition, alterations in polyamine synthesis and release have been proposed as a potential mechanism for ethanol dependence and withdrawal [21]. Polyamines also modulate other ligand-receptor interactions, including estradiol binding to estrogen receptor (ER) [22].

Recent studies indicate that protein-protein interactions are altered by polyamines. We found that inhibition of polyamine biosynthesis drastically reduced the number of proteins associated with ER [23]. Addition of spermidine enhanced ER-association of proteins of molecular weights 300, 180, 160, 140, 100, 80, 60, 45, 35, and 30 kD. Interestingly, two proteins of estimated molecular weights 100 and 160 kD, increased in intensity at 500 μM spermidine, but decreased at 1000 μM spermidine [23]. Maeda et al [24] reported that spermine enhanced the interaction of a coactivator, DRIP205 (vitamin D receptor interacting protein) to vitamin D receptor (VDR), but decreased the interaction of VDR to another coactivator, GRIP1 (glucocorticoid interacting protein). These authors proposed that spermine might act as a switch in choosing the coactivator interactions of nuclear receptors. They also suggested that the unravelling of chromatin may provide a localized source of polyamines, as they might be dissociating from their chromatin binding sites during transcription.

Gene regulation by polyamines may also have components that are not well recognized. Thus, acetylation of polyamines is usually considered as a first step toward their degradation [5]. However, acetylation of spermidine provides an additional chance for bifurcation of polyamine function. Acetylation of the nitrogen adjacent to the 3-carbon chain yields N^1-acetylspermidine, whereas acetylation of nitrogen adjacent to the 4-carbon chain produces N^8-acetylspermidine. The latter form of acetylated spermidine does not lead to its conversion to putrescine, but undergoes deacetylation in the presence of N^8-acetylspermidine deacetylase [25]. An example of an independent action of N^8-acetylspermidine was recently reported on PC12 cells which differentiated in its presence with neurite growth and dopamine production, whereas spermidine and N^1-acetylspemidine did not produce these changes [26].

In addition to the direct effects of polyamines on gene regulation through DNA-protein and protein-protein interactions, there is evidence for polyamine involvement in different stages of signal transduction. For example, the activity of purified casein kinase II increased by 2- to 20-fold in the presence of polyamines [27]. Another instance of polyamines acting as signalling molecule is observed in using rat smooth muscle cells, when cytostatic effect of difluoromethylornithine

(DFMO) could be prevented by MAP kinase 1/2 inhibitor [28]. In these cells, addition of DFMO leads to activation of p42/p44 MAPK and induction of $p21^{Waf1/CIP1}$. This protein is an inhibitor of cell cycle progression as it inhibits the action of cyclin/cdk complexes required for cell cycle progression [29]. In L1210 cells, MAP kinase is reported to activate ODC [30]. Therefore, decreased levels polyamines can lead to activation of MAP kinase and this activation can lead to increased ODC activity and increased levels of polyamines. ODC overexpressing breast epithelial cells (MCF-10A) exhibited increased MAPK phosphorylation in response to epidermal growth factor [31]. Taken together, there are strong links between ODC, polyamines and MAPK kinase pathway, although the regulatory loops may differ in different cell types.

Polyamine synthesis, metabolism, uptake and function can be used as targets for cancer prevention and therapy. Research on different components of polyamine metabolism and function relevant to cancer therapy are summarized below.

Ornithine decarboxylase (ODC)

ODC has long been known as a marker of carcinogenesis and tumor progression [32]. Studies demonstrating that overexpression of ODC gene in NIH 3T3 cells leads to transformation of these cells support a role for ODC and polyamines in the origin and progression of neoplastic diseases [33,34]. Blockade of polyamine biosynthetic pathway can be achieved by the inhibition of ODC. Thus, an irreversible inhibitor of ODC, DFMO was synthesized in 1978 [35]. Studies using DFMO demonstrated the essential nature of polyamines in many physiological functions in the cell (36). Cancer cells undergo cytostasis in the presence of DFMO and this growth arrest can be prevented by the treatment of cells with putrescine [35-37]. While compensatory pathways in the polyamine biosynthesis and increased cellular uptake of polyamines dampened the effectiveness of DFMO as a singular cancer therapeutic agent, it is currently undergoing clinical trials for cancer prevention [38,39]. The effects of DFMO in combination chemotherapy has not been fully developed.

Transgenic mice overexpressing ODC provide insights into the role of ODC and polyamines in the carcinogenesis process. Aberrant expression of ODC alone was not sufficient to induce tumors in fibroblasts or keratinocytes [40]. However, oncogenes cooperate with ODC in inducing transformation [41,42]. ODC transgenic mice produce skin tumors with the administration of carcinogen alone, while normal mice would require the administration of carcinogens and a tumor promoter [43,44]. These results demonstrate that increased levels of cellular polyamines due to the overexpression of ODC contribute to carcinogenesis and tumor progression.

Studies on cancer susceptibility genes support the role of ODC as a modifier of the transformation process. Familial adenomatous polyposis (FAP) arises following mutation or loss of adenomatous polyposis coli (APC) gene [45]. Alterations of the APC tumor suppressor gene in germline cells of rodents and humans is associated with increased intestinal activity of ODC [46]. In another cancer syndrome, Xeroderma pigmentosum (XP), patients are deficient in nucleotide excision repair (NER) because of mutations in one of the nucleotide excision repair (NER) genes, resulting in high frequency of UV-induced tumors [47]. In a mouse model of Xp (Xpa knockout mice), treatment with DFMO prevented the outgrowth, but not the initiation, of UV-induced tumors [48]. Thus, ODC remains as an important target of intervention and therapy, even in the case of cancer incidence due to alterations in cancer susceptibility genes.

S-adenosylmethionine decarboxylase (SAMDC)

The first attempt to target the polyamine biosynthetic pathway for cancer therapy was as early as 1972, when the first SAMDC inhibitor, methylglyoxal bis(guanylhydrazone) (MGBG), was synthesized [49]. However, MGBG was not very specific for SAMDC and was found to exert mitochondrial toxicity [50]. A combination of MGBG and DFMO was found to be highly toxic in prostate cancer patients without therapeutic response [51]. These results dampened enthusiasm for utilizing SAMDC inhibitors for cancer therapy. However, there is a renewed interest in utilizing SAMDC inhibitors for

cancer therapy [52,53]. A new generation of SAMDC inhibitors were synthesized and tested in different tumor models. Results of a Phase I trial on one of these inhibitors, CGP 48664 or SAM468A, was published recently [52]. Although no complete or partial response was reported in this study, safe administration of the drug was demonstrated with stable disease in adrenal, renal, sarcoma and head and neck cancers. A maximal tolerated dose of 102.4 mg/m^2/day was established.

While data on the role of polyamines from different laboratories and experimental models may include confounding factors, an intriguing study of the consequences of SAMDC overexpression or blockage of its synthesis exemplifies a paradox on polyamine function [54]. NIH 3T3 cells engineered to overexpress SAMDC in the sense or antisense orientation were transformed and the transformed cells were able to induce tumors in nude mice [54]. This study demonstrate the delicate requirement of balance in polyamine biosynthesis and the control of intracellular polyamine levels in avoiding malignant cell growth. Examples of bimodal interactions of polyamines are often found in *in vitro* studies, where interactions of polyamines with DNA, or in the modulation of ligand-receptor binding, or protein-DNA binding are examined [4]. Thus a ligand-receptor interaction may be facilitated at a narrow concentration range, followed by inhibition of the interaction at a higher concentration [55]. This requirement for balance of polyamines for the growth of normal cells and the wellbeing of the organism is an impedient in the application of polyamine-based drugs for cancer therapy since drastic reduction in polyamine levels will invariably lead to toxicity.

Spermidine/ spermine synthase

Spermidine synthase and spermine synthase are enzymes responsible for the synthesis of spermidine and spermine, respectively [56]. They are also known as putrescine aminopropyl transferase (PAPT) and spermidine aminopropyl transferase (SAPT). The reactions catalyzed by PAPT and SAPT are similar, except for the utilization of putrescine by PAPT and spermidine by SAPT. Attempts to synthesize inhibitors of PAPT and SAPT have resulted in cyclohexylamine for PAPT and N-(n-butyl)-1,3-diaminopropane (BDAP) and N-(3-aminopropyl) cyclohexylamine (APCHA) for SAPT [57,58]. S-adenosyl-1,8-diamino-3-thiooctane (AdoDATO) is a more potent and specific inhibitor of PAPT, with no inhibitory effect on SAPT [59]. In contrast, S-adenosyl-1,12-diamino-3-thio-9-azadodecane (AdoDATD) is a potent inhibitor of SAPT with weak inhibitory activity on PAPT [60]. Exposure of mammalian cells with AdoDATO resulted in a drastic decrease in cellular spermidine, with a concomitant increase in putrescine, consistent with the inhibition of spermidine synthesis [61]. Interestingly, there was an increase in spermine as well. AdoDATO concentrations that produced maximal decrease in spermidine levels resulted in a significant decrease in cell number. Similarly, exposure of rats with the PAPT inhibitor, trans-4-methylcyclohexylamine for 10 days led to 70-80% reduction in spermidine in a variety of tissues; however, there was a compensatory increase in spermine, sustaining cell growth [62].

Treatment of HT-29 and L1210 cells with AdoDATD resulted in a decrease in the level of spermine, which was compensated by an increase in the level of spermidine [63]. Huber and Poulin [64] showed antiproliferative activity of SAPT inhibitor, APCHA and that the growth inhibition was reversible by the addition of spermine. Interestingly, APCHA potentiated the action of DFMO. This is important because the action of DFMO is often compromised by an up-regulation of spermine. Prolonged exposure of rats with APCHA led to a 50% decrease in spermine content in the liver, without major effects on the weight of the animals. Thus, organisms have intricate control mechanisms for maintaining total polyamine pools when inhibitors are used to reduce the level of any one of the enzymes in polyamine biosynthesis. Consequently, it might be necessary to define the abnormalities in polyamine metabolism of cancer cells in order to effectively deal with the defect and utilize inhibitors in cancer therapy.

Spermidine/spermine acetyl transferase (SSAT)

SSAT is a well characterized polyamine catabolic enzyme [5]. Acetylation of spermidine and spermine reduces the potency of the polyamine as the

positive charge on the amino terminus is partially neutralized [4,65]. Acetylation also marks the polyamine for degradation by polyamine oxidases. While SSAT protects cells from excessive levels of polyamines, it can also cause injury, due to polyamine degradation products such as acetoamidopropanal and hydrogen peroxide [5,66]. Often, SSAT is a marker for polyamine degradation and depletion in response to an increase in the level of natural polyamines, treatment with polyamine analogues, cellular oxidative stress or other injury [67-69]. Furthermore, polyamine acetylation may be linked to histone acetylation, and certain histone acetylases may have the ability to acetylate polyamines as well [70]. Since increased histone acetylation has been found in ODC overexpressing cells [71], it is possible that regulation of a network of genes is controlled by the co-ordinated action between histone acetylation, polyamine levels and polyamine acetylation.

Recent studies using SSAT overexpressing transgenic mice might help to define the role of SSAT in polyamine homeostasis [72,73]. SSAT overexpressing mice showed large increase in tissue levels of putrescine, and the magnitude of this increase was larger than those found in ODC overexpressing transgenic mice. Disturbances in polyamine pools was accompanied by phenotypic changes such as hair loss, skin wrinkling, loss of subcutaneous fat, and in females, underdeveloped uterus and abnormal ovaries [72]. Targeted overexpression of SSAT in the epidermal keratinocytes (K6-SSAT transgenic mice) led to phenotypically normal mice, with increased susceptibility to DMBA/TPA-induced skin tumors [74]. These mice showed a 10-fold increase in the number of epidermal tumors that developed in response to the carcinogen/promoter treatment. Furthermore, SSAT overexpressing animals were highly sensitive to polyamine analogues due to increased catabolism and excretion of polyamines [75].

SSAT deficient embryonic cells have provided further insights into the connections between polyamine homeostasis and SSAT [76]. As can be expected from the increased susceptibility of SSAT overexpressing transgenic mice to polyamine analogues, SSAT deficient cells were more resistant to polyamine analogues. Surprisingly, this resistance was not due to a lack of polyamine depletion since wild type and SSAT deficient cells had similar levels of polyamine depletion. This result supports previous studies on polyamine depletion due to the inhibition of ODC and SAMDC by polyamine analogues, independent of the presence of SSAT. The lack of SSAT may reduce the chances of polyamine degradation and the production of hydrogen peroxide and thereby the cytotoxicity of the polyamine analogue. However, cell type dependent compensatory pathways of polyamine homeostasis are not fully elucidated. The cloning of a novel spermine oxidase indicates an alternate pathway for the interconversion of polyamines [77].

Polyamine oxidases

Oxidation of polyamines leads to the back-conversion of spermine to spermidine, and spermidine to putrescine. Acetylated polyamines are generally the preferred substrates of polyamine oxidase (PAO), a flavin containing amine oxidase present in all vertebrate tissues [78]. PAO catalyzes the oxidation of spermine and spermidine via an oxidative cleavage of the α- CH bond of the substrate to form an imine product with concomitant reduction of the flavin cofactor [79]. The imine product is then hydrolyzed to the corresponding aldehyde and ammonia. The reduced flavin coenzyme reacts with oxygen to form hydrogen peroxide and the oxidized form of the flavin. These "by-products" of polyamine degradation may impart characteristic changes in redox signalling, leading to modulation of cell proliferation or apoptosis [80,81]. Polyamine oxidase is reported to be induced in response to treatment of lung carcinoma cells with polyamine analogues [82]. In addition, a polyamine oxidase inhibitor (MDL-72,527) exerted apoptotic effects through the depletion of putrescine and spermidine and the formation of numerous lysosomally derived vacuoles [83]. Thus, the action of polyamine oxidase seem to be important to polyamine homeostasis and the regulation of this enzyme may have a role in facilitating apoptosis of cancer cells.

Comparison of the crystal structures of a plant PAO and human monoamine oxidase (MAO), which degrades neurotransmitters, shows struc-

tural similarities with 20% amino acid sequence identity [84]. However, PAO and MAO differ substantially in the overall topology of their substrate binding sites. The catalytic site of PAO is lined with several acidic amino acid residues which may steer the polyamine substrates to the binding site. Polyamines are also broken down by diamine oxidase which is a copper/quinone containing serum amine oxidase [84,85]. This enzyme is generally responsible for the cytotoxicity of polyamines in cell culture models in the presence of fetal calf serum. Although the physiological role of this enzyme is not known, it is believed to impart immunosuppressive and bacteriocidal actions [86]. During pregnancy, there is a marked induction of serum amine oxidase which is correlated to protection against spontaneous abortion [87]. Cloning of a novel mammalian spermine oxidase (SMO), which preferred spermine over acetylated spermine as the substrate as well as characterization of multiple splice variants of PAO are new developments in this area [77,88].

Polyamine transport as a target

Increased efficacy of DFMO was seen in animal models of cancer when a polyamine-free diet was provided [89,90]. This result suggested that DFMO-mediated polyamine depletion is, in part, compensated by polyamine uptake from dietary components. Therefore, compounds that prevent polyamine transport can be effective in augmenting the anti-proliferative activity of DFMO. Early attempts to develop compounds to inhibit polyamine transport led to a number of polypyridinium salts, based on the finding that paraquat (4'4'-bipyridine) is transported by the polyamine uptake pathway [91-93]. Poulin and collaborators synthesized 2,2'dithiobis(N-ethyl-spermine-5-carboxamide (DESC) and its thiol monomer as polyamine transport inhibitors [94]. Although DESC strongly decreased the initial rate of [^{3}H]-spermidine transport, even a 40-fold molar excess of the compound did not completely inhibit spermidine accumulation. These investigators also synthesized dimers of spermidine or norspermidine and demonstrated their activity as polyamine transport inhibitors using T-47D breast cancer cells [95]. Aziz *et al.* [96] synthesized a 25 kDa polymeric spermine and showed its ability to inhibit polyamine uptake. These different transport inhibitors require further studies to demonstrate their therapeutic utility individually or in combination with DFMO under *in vivo* conditions.

Recently, Burns *et al.* [97] described the synthesis and characterization of a series of spermine/ amino acid conjugates. The presence of the amide group enhanced the affinity of the compound for the polyamine transporter. A potent compound was a lysine-spermine conjugate which showed anti-proliferative activity in combination with DFMO. Interestingly, this compound was able to inhibit the growth of DFMO-treated cells even in the presence of exogenous spermidine. Using comparative molecular field analysis (CoMFA), Li *et al.* [98] constructed a model to correlate molecular structure with inhibition of [^{3}H]-spermidine uptake. The CoMFA model successfully predicted the inhibitory potency of polyamine analogues that had not been previously tested. The test group included aziridinyl diamines, acetylated spermidine, two new oxazolidinonyl spermidine, monoaziridinyl spermidine, and a diaziridinyl spermine. Some of these compounds had anti-tumor activity in mice.

Polyamine transport mechanisms are not well characterized in mammalian cells, although transport is known to be energy-dependent and saturable, suggesting a carrier mediated process [99]. Several proteins that regulate polyamine transport are known. Genes encoding protein kinases have been reported to be required for enhanced uptake of polyamines in yeast cells [100, 101]. On the other hand, ODC antizyme down-regulates polyamine uptake [102]. A recent study indicates that the uptake of agmatine, a biogenic amine that links polyamine biosynthesis pathway and the generation of nitric oxide, utilizes polyamine transport system [103]. Three genes coding for transport proteins have been cloned from *E. Coli* [104]. Two of these genes are specific for putrescine or spermidine, while a third is involved in the excretion of putrescine by putrescine-ornithine anti-porter activity. Better characterization of the polyamine transport proteins in mammalian cells may facilitate the uti-

lization of polyamine uptake characteristics in the design of novel anti-cancer drugs.

ODC antizyme

ODC antizyme is another component in the regulation of polyamine homeostasis [99]. ODC is often described as one of the most labile enzymes, and yet it is not marked by ubiquitination for degradation by the proteosome [105]. Instead, the binding of ODC antizyme, a small protein synthesized in response to increased levels of polyamines, facilitates the rapid degradation of ODC by the 26S proteosome [106]. In addition to the down-regulation of polyamine biosynthesis due to the degradation of ODC, antizyme also suppresses polyamine transport system (102). This dual role of antizyme underscores its importance as a link between abnormal polyamines levels and tumorigenesis [107-109]. Recent studies indicate defective regulation of antizyme in oral and prostate cancers [107,108]. In addition, increased antizyme production in melanoma cells is associated with growth inhibition [109]. Induction of antizyme is also an important factor in the anti-proliferative activity of polyamine analogues [110].

Antizyme belongs to a conserved family with at least three members in the vertebrates [106]. Antizyme 1 and antizyme 2 are generally present in all tissues, with antizyme 1 found in 10- to 20-fold excess compared to antizyme 2 [111]. Antizyme 3 is found only in testes and is expressed during postmeiotic stages of spermatogenesis [112]. Translation of antizyme mRNA requires an unusual polyamine-dependent +1 frame shift [106]. Studies on FM3A mouse cells indicate that antizyme not only suppresses polyamine uptake, but it also stimulates the excretion of polyamines to the culture medium [113].

Polyamine analogues

While it has been difficult to develop polyamine transport inhibitors, the promiscuity of transport pathway in allowing the uptake of many structurally similar compounds has been at the heart of developing polyamine analogues for cancer therapy [114, 115]. Many of the analogues appear to displace natural polyamines since there is a tendency to deplete natural polyamines and yet maintain relatively constant level of total polyamine pools. As polyamine analogues are unable to carry out the functions of natural polyamines in the cell, there is a collapse of the cellular functions, as indicated by programmed cell death or apoptosis. Since cancer cells have higher levels of natural polyamines and increased requirement and/or defective regulatory mechanisms [4, 5], they can be expected to be more sensitive to the action of polyamine analogues than normal cells.

Traditional polyamine analogues have been designed by altering the chain-length of methylene groups bridging primary and secondary amino groups, introducing substituents at various positions, and by alkylating the amino end groups [114,115]. Porter *et al*. [116] synthesized bis(ethyl) derivatives of putrescine, spermidine and spermine. Among these compounds, bis(ethyl)spermine analogues were more effective anti-proliferative agents than putrescine or spermidine derivatives. Subsequent developments included asymmetrically alkylated polyamine analogues, dimethylsilane compounds or conformationally constrained polyamine analogues [117-119].

Elucidation of the mechanism of action of polyamine analogues remains elusive, although each of the polyamine functions and interactions are possible targets of their action. Direct action of polyamine analogues with DNA remains to be a major pathway in the mechanism of action of polyamine analogues [120]. Polyamine analogues might interfere with DNA-polyamine interactions and cause problems in condensation, packaging of DNA and its unravelling during transcription and replication [4,121]. As a result, analogues may alter cell cycle regulatory mechanisms, including the induction of cyclin B1, cyclin D1, p53 and cyclin dependent kinase inhibitors [122-124]. These changes in gene expression may occur due to interactions of the analogues with DNA, interference with transcription factor-DNA interactions or by altering the stability of mRNAs [125,126]. In the case of certain asymmetrically alkylated polyamine analogues, anti-mitotic effects of these compounds were attributed to their ability to interfere with tubulin polymerization [119].

Bis(ethyl)polyamine analogues induce apoptosis of tumor cells, although no single mechanism can explain all of the effects of polyamine analogues [17,81,83,124]. Polyamine analogues may alter the expression of genes associated with apoptotic pathways. Bis(ethyl) (BE) polyamine analogues are often abbreviated by the number of methylene groups separating the primary and secondary amino groups. Analogues BE-3-4-3, BE-3-3-3 and BE-4-4-4 decreased the expression of the c-*myc* oncogene and protein levels in cancer cells [125]. The anti-apoptotic protein bcl-2 was suppressed by BE-3-3-3 in MCF-7 breast cancer cells [17]. Treatment of human melanoma cells with BE-3-3-3 led to the loss of mitochondrial transmembrane potential at 24 h, indicating the activation of mitochondrial apoptotic signaling pathway [126]. In other cases, degradation products of polyamine analogues might be the inducers of apoptosis [81]. In either case, the non-toxic treatment window is narrow, because of the multiple functions of polyamines in the cell and in the organism. However, the higher sensitivity of some cancer cells to apoptosis by chemotherapeutic agents might be utilized in the case of polyamine analogues, with careful pre-clinical and clinical research.

Future directions

Multiple pathways for the control of polyamine synthesis, catabolism and uptake suggest that cells might be able to recover and maintain polyamine homeostasis, if there is just one defect in the regulatory network. Multiple defects such as overexpression of ODC and deficient antizyme production might coexist in cells containing elevated polyamine levels. Therefore, complete analysis of the regulatory pathway in cancer cells and adjacent normal cells, using a comprehensive microarray/ proteomic approach will be helpful to map out the defects in polyamine metabolism in a specific type of cancer. Understanding the defects in the regulatory pathways will also facilitate the selection of the most effective chemotherapeutic agents and polyamine anti-metabolites for the treatment of a particular type of malignancy.

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