The Role of Polyamines in Human Cancer: Prospects for Drug Combination Therapies

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Abstract

Ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC) are two key enzymes in polyamine (PA) biosynthesis and their inhibition leads to PA pool depletion and cell growth arrest. DFMO and SAM486A are specific inhibitors of ODC and AdoMetDC, respectively, and are the only two PA inhibitors, which have been clinically evaluated in Phase II and III cancer trials. However, drug combination therapies expected to potentiate the effects of these drugs have yet to be systematically pursued. Human cancer trials (e.g. for the treatment of neuroblastoma patients) using a DFMO/ SAM486A cocktail, possibly combined with current cytotoxic drugs and concomitant with a PA-deficient diet, are warranted.

Polyamine biosynthesis in mammalian cells

The naturally occurring polyamines (PAs) are small aliphatic cations identified over three centuries ago. PAs are found in all living cells and are responsible for a plethora of functions including cell growth, differentiation, apoptosis, and DNA replication.[1-3] Mammalian cells produce the PAs putrescine, spermidine, and spermine.[2,4] The diamine putrescine is formed from ornithine via the action of ornithine decarboxylase (ODC), a key enzyme in PA biosynthesis (Fig. 1). Putrescine can be further converted into the higher PAs spermidine and spermine. The aminopropyl groups necessary for these conversions are provided via decarboxylation of S-adenosylmethionine (AdoMet) to decarboxylated S-adenosylmethionine

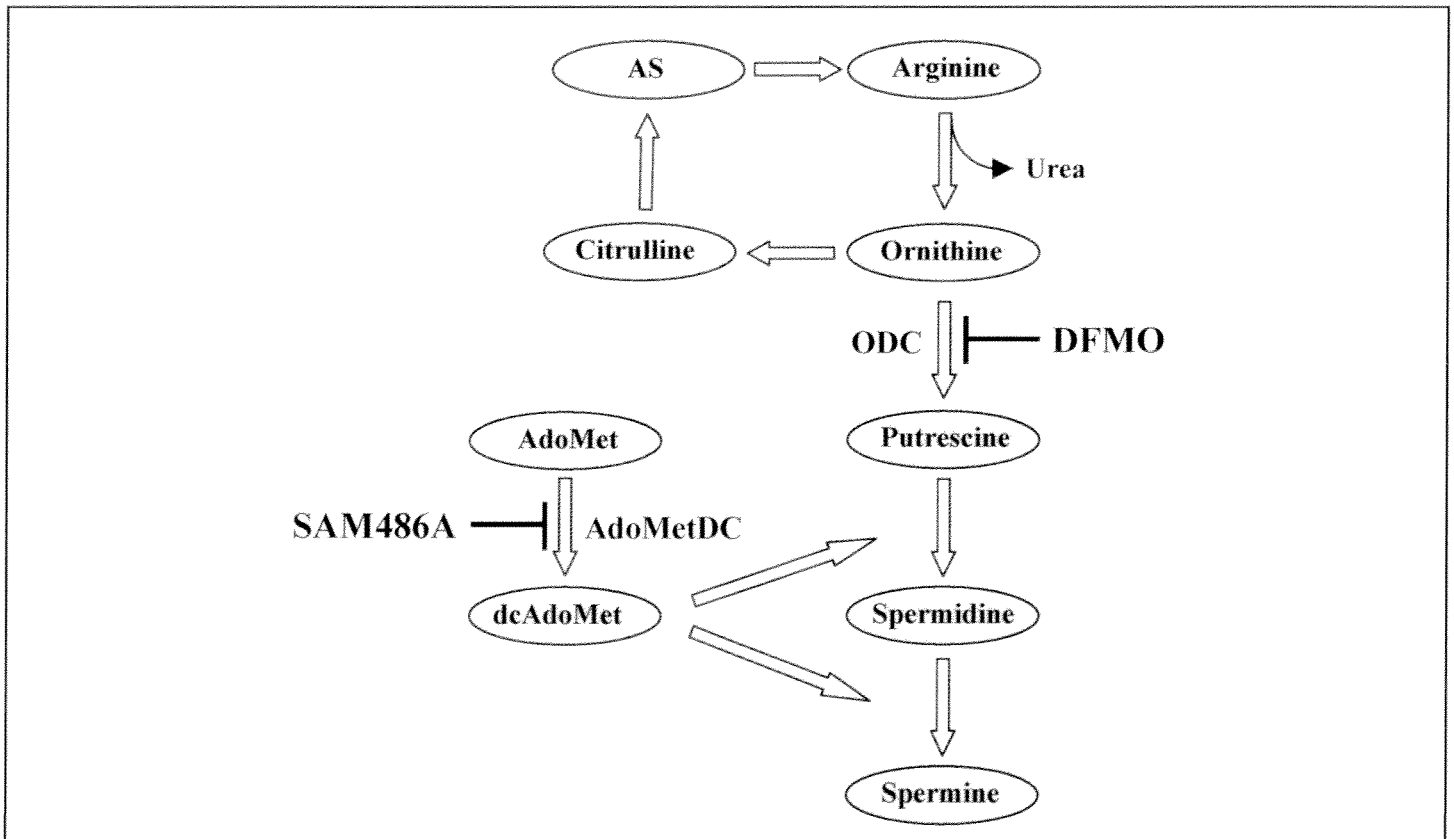

Figure 1.— Simplified diagram of the polyamine (PA) biosynthetic pathway and associated amino acids of the urea cycle showing key enzymes ODC and AdoMetDC and their specific inhibitors DFMO and SAM486A, respectively. Abbreviations are: AdoMet, S-adenosylmethionine; AdoMetDC, S-adenosylmethionine decarboxylase; AS, argininosuccinate; DFMO, α-difluoromethylornithine (also known as Eflornithine); ODC, ornithine decarboxylase; SAM486A, 4-amidinoindan-1-one 2'-amidinohydrazone (also known as CGP48664A).

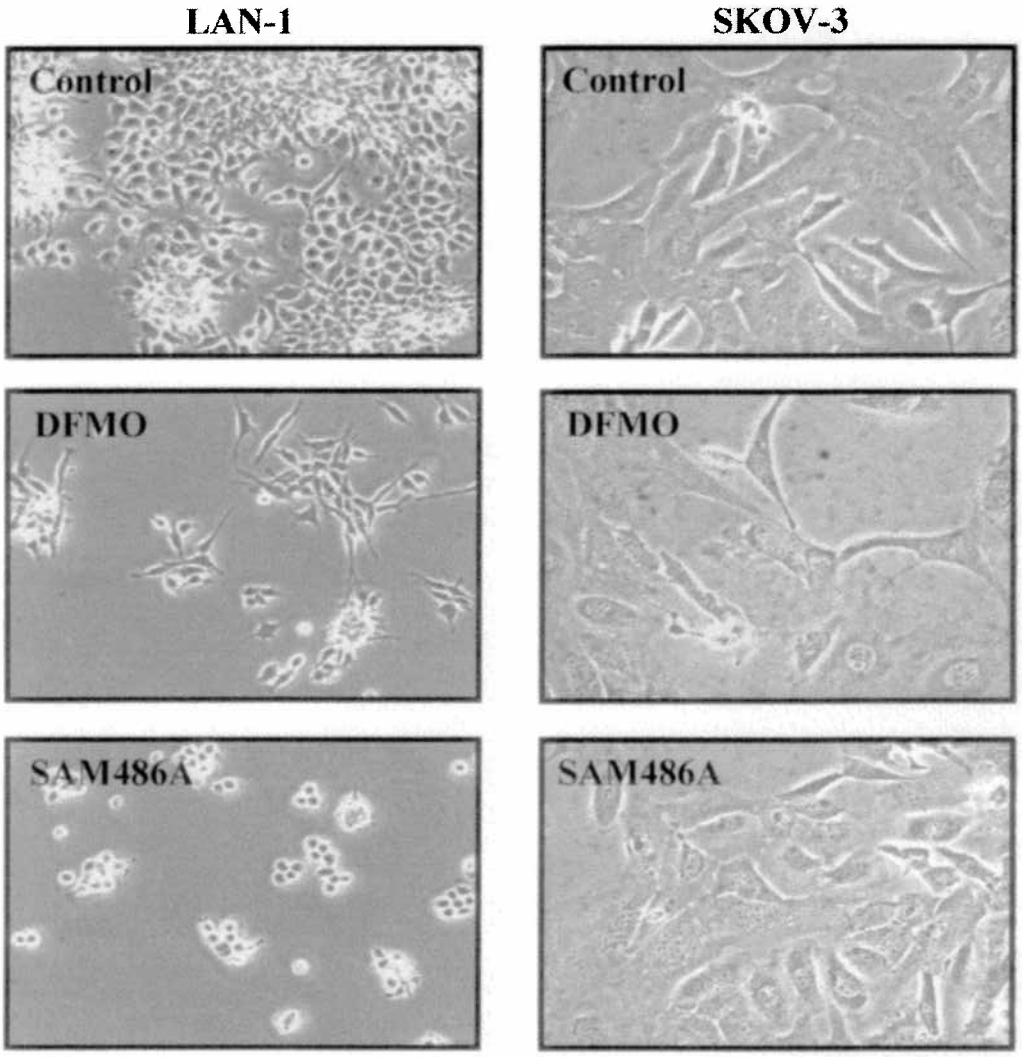

Figure 2.— Effect of 5 mM DFMO or 10 μM SAM486A on the growth of MYCN-amplified and p53 mutant human neuroblastoma (NB) cell line LAN-1 and human ovarian cancer cell line SKOV-3. Cells were treated without or with indicated polyamine (PA) inhibitors DFMO or SAM486A for 3 days. Micrographs were taken using an inverted phase contrast microscope (Nikon). In comparison with the SKOV-3 ovarian cancer cells, the growth of LAN-1 NB cells was inhibited much more drastically and caused significant morphological changes after only 3 days of treatment. This suggests that NB cells are more responsive to PA inhibitors and that the clinically evaluated drugs DFMO and SAM486A might provide an alternative approach for the treatment of NB patients.

(dcAdoMet) (Fig. 1).[5] The positively charged PAs allow for both electrostatic and hydrophobic interactions with DNA, RNA, and proteins, thereby directly affecting gene regulation. There is also increasing evidence that PAs are involved at various stages of signal transduction, and, for example, regulate and phosphorylate important cellular components of the MAPK and PI3K signaling pathways[6-8] (our results, unpublished).

Polyamines are highly regulated during cell cycle progression

Normal cell growth is orchestrated in a cyclic manner by the action of cyclins and cyclin-dependent kinases (cdks)[9] and appropriate activation/ inactivation of these proteins is necessary for cell cycle progression. The cyclins A, B, D, and E form complexes with corresponding cdks and specifically regulate the G_1/S and G_2/M phases of the cell cycle. Similarly, ODC and PA concentrations increase in both cell cycle phases.[10,11] This strong positive relationship to cell cycle regulation provides further evidence that PAs are intrinsically linked to cell growth and proliferation.[12]

Elevated polyamine levels can lead to cancer

PA levels are elevated in many types of cancer, and interference with PA biosynthesis has long been considered a promising therapeutic approach against proliferative diseases, including various malignancies.[13-22] Elevated PA levels have also been detected in urine of cancer patients and can be measured in blood and cerebrospinal fluids.[11,16,23] ODC has been known as a marker of carcinogenesis and tumor progression [24] and has been considered a proto-oncogene,[25] which is transactivated by c-Myc.[26] The high enzymatic activities of ODC and AdoMetDC in rapidly growing cells and tissues, and especially,

in tumor cells, rendered a rationale for designing pharmacological inhibitors, which selectively interfere with the natural biosynthesis of PAs and, consequently, prevent tumor cell growth.

The clinically established polyamine inhibitors DFMO and SAM486A

Over the course of more than 30 years, many researchers have focused on the design and synthesis of inhibitors, which interfere with PA metabolism.[11,18-20] A large number of these synthetic inhibitors and PA analogues were evaluated *in vitro*, in cell culture experiments, and in animal cancer models. However, so far, only two PA inhibitors have further advanced to the clinical setting. The most prominent drug is the ODC inhibitor α-difluoromethylornithine (DFMO; also known as Eflornithine) (Fig. 1), which has been evaluated in Phase II and III cancer trials.[27-29] The second drug is the AdoMetDC inhibitor 4-amidinoindan-1-one 2'-amidinohydrazone (SAM486A; also known as CGP48664A) (Fig. 1), which was assessed in Phase I trials,[30,31] and most recently, in a Phase II multicenter study.[32] Although monotherapy with DFMO has been disappointing in most cancer trials, the drug was found more effective as a chemopreventive agent based on its low toxicity. The reported side effects are relatively mild with occasional occurrence of temporary ototoxicity, diarrhea, and some neutropenia. Notably, DFMO is successfully used in the treatment of a number of parasitic diseases, including the infection with *Trypanosoma brucei gambiense*, which causes African trypanosomiasis.[33] Recent Phase II clinical trials with SAM486A in patients with relapsed or refractory non-Hodgkin's lymphoma were promising and the most frequent side effects included nausea, vomiting, diarrhea, asthenia, abdominal pain, and flushing.[32]

DFMO and SAM486A inhibit growth of human neuroblastoma cells

Because DFMO and SAM486A are the only clinically evaluated PA inhibitors with a relatively high tolerance in patients, we have focused our own research on these two drugs and are studying their effects on human neuroblastoma (NB) cells. NB is an extra-cranial tumor in infants and originates from precursor cells of the peripheral (sympathetic) nervous system and usually arises in a paraspinal location in the abdomen or chest.[34] We found that NB cells respond more rapidly and more profoundly to the growth inhibitory effects of DFMO and SAM486A than, for example, ovarian cancer cells or other cell lines discussed in the literature (Fig. 2). To our knowledge, NB trials with DFMO or SAM486A have not been conducted thus far, and, given the need for new anti-neoplastic agents with novel mechanisms of action, such trials should be seriously considered. Our research further revealed that DFMO and SAM486A are effective against NB cells with *MYCN* amplification (typically derived from more aggressive NB tumors, which metastasize and do not respond well to conventional chemotherapy) and with mutated tumor suppressor protein p53 (often found in relapsed and chemoresistant NB tumors), thus further supporting the use of these drugs for therapeutic NB treatments.

Prospective combination therapies for improved efficacy in cancer trials

Although ODC has generally been considered as the enzyme catalyzing the rate-limiting step in PA biosynthesis, it has been shown that the supply of dcAdoMet represents a second rate-limiting factor in PA biosynthesis,[35] and therefore, the enzyme AdoMetDC represents a second rational target (Fig. 1). Since the two enzymes are co-regulated by intracellular PA pools so that inhibition of one results in a compensatory increase in the other, it follows that targeted interference with a drug cocktail composed of ODC inhibitor DFMO and AdoMetDC inhibitor SAM486A (or other prospective PA inhibitors of clinical relevance) is likely to sharpen the antiproliferative effects by complete depletion of the PA pools. Such combination therapies could be further enhanced by the inclusion of retinoic acid (RA), a well-characterized agent that induces neuronal cell differentiation and is used in NB therapy.[36] In addition, RA affects PA levels and inhibits ODC activity,[1,20,37,38] thus further contributing to the total depletion of PA pools. Finally, the combination of the cytostatic drug DFMO with SAM486A and some of the current cytotoxic drugs may provide a more powerful regimen against NB and other cancer types.

Conclusions

PAs are intrinsically connected to cell growth and proliferation, and the inhibition of the sentinel PA biosynthetic enzymes ODC and AdoMetDC is considered a means to prevent cell proliferation by PA depletion, the blockade of cell cycle progression, and interference with signal transduction. DFMO and SAM486A have both been evaluated in human cancer trials, but so far, have only been studied individually. Thus, further studies assessing the efficacy of combined drug therapies are certainly warranted. The treatment of NB patients with a DFMO/ SAM486A cocktail, possibly in combination with RA and concomitant with a PA-deficient diet should be considered as an alternative protocol.

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