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Emerging Concepts in Targeting the Polyamine Metabolic Pathway in Epithelial Cancer Chemoprevention and Chemotherapy

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The polyamines are important molecules governing cell proliferation, survival and apoptosis. Consistent with their elevated levels in cancer, they have been shown to mediate tumor promotion and progression. Cellular and tissue polyamine pools and metabolic flux are regulated by a number of processes. Neoplastic transformation is accompanied with an increase in biosynthesis, decreased catabolism and elevated uptake of exogenous polyamines. Effective strategies for cancer chemoprevention and chemotherapy, targeting the polyamine metabolic pathway will likely require a combination of agents acting at multiple sites of this pathway. Genetic variability affecting expression of the ornithine decarboxylase gene suggests an association between ODC expression and cancer risk, and prediction of response to treatment in certain epithelial cancers.

Key words: chemoprevention, c-MYC, DFMO, MAD1, ODC.

Ever since their discovery in 1678 by Leeuwenhoek, polyamines have intrigued the physicist, the chemist and the biologist (*1*). These ubiquitous polycations are found in all living organisms, except two orders of Archaea (*2*). Putrescine, spermidine and spermine are the most important polyamines from the biologist's point of view. These cations are highly multifunctional and have been known to be involved in a variety of processes governing cell proliferation and survival (*3*). They have been known to be involved in affecting DNA structure by virtue of their positive charge and in turn, influence gene expression. They have also been known to regulate cell proliferation and apoptosis, ion channels, and cell signaling (*4*).

Intracellular polyamine levels is an important determinant of cell viability, an increase or a decrease both leading to apoptosis (*5*). Thus, it is clear that intracellular polyamine contents need to be stringently controlled for optimal functioning of the cell. Intracellular polyamine content is tightly regulated at the levels of biosynthesis, catabolism, uptake and efflux. The polyamine metabolic pathway has been extensively studied (for a recent review refer to Ref. *6*). Briefly, polyamines are obtained either through biosynthesis or through uptake from extracellular sources. Biosynthesis is mediated by the enzyme ornithine decarboxylase (ODC), which converts the amino acid ornithine into the diamine putrescine. Putrescine is then sequentially converted into the tri- and tetraamine spermidine and spermine respectively, through the action of the enzyme *S*-adenosylmethionine decarboxylase (AdoMetDC) and spermidine synthase/spermine synthase. Uptake of polyamines in mammalian cells has not been elucidated. Work done by Belting *et al.* (*7*), as well as in our lab (U. Basuroy and E.W. Gerner, unpublished observations) allude to an endocytosis-dependent mechanism for uptake. Polyamine levels are also regulated by catabolism. The central enzyme in this process is the spermidine spermine–*N*-acetyl transferase enzyme (SSAT), which adds acetyl groups to terminal amine groups in spermidine and spermine. These acetylated polyamines are then substrates for the enzyme polyamine oxidase which retroconverts these acetylated derivatives into lower chain amines. These acetylated derivatives can be exported out of the cell. Xie *et al.* have characterized one such mechanism of polyamine export (*8*). They have shown that this "diamine exporter" can be effectively blocked with agents like verapamil.

The protein ornithine decarboxylase antizyme (OAZ), whose translation depends on intracellular polyamine levels, is central to polyamine homeostasis (*9*). Accumulation of polyamines triggers the unique +1 frameshift of the translating ribosomes on the anzityme mRNA, leading to increased translation of the OAZ protein. OAZ can effectively control polyamine levels by inactivating ODC and inducing its degradation, increasing polyamine efflux and decreasing polyamine uptake. Thus, the OAZ mRNA/translation module is an effective polyamine sensor which keeps intracellular polyamine levels in check. Recently, a novel inhibitor of the OAZ, antizyme inhibitor or AZI has been characterized (*10*). The protein is an inactive form of ODC which competes with ODC for binding to OAZ, and thus sequesters OAZ and prevents it from binding to ODC. Overexpression of AZI in certain forms of cancer has been recently reported.

At this stage, a mention must be made of the eukaryotic initiation factor, eIF5A. This molecule is intricately linked to the polyamine pathway because its activity is governed by a unique post-translational modification dependent on cellular spermidine levels (*11*). This process is initiated by the addition of a spermidine molecule to a lysine residue, resulting in the formation of an unique amino acid, hypusine. The hypusine is then dehydroxylated in a second step,

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to give the active form of eIF5A. The eIF5A protein is closely related to one of the restriction points of the cell cycle (*12*).

Polyamines and cancer: Cause or consequence?

Polyamines regulate important cellular processes including apoptosis and proliferation. Both these are extremely crucial in determining cell turnover and hence, play an important role in cancer development. Though the function of these amines had been extensively studied in other diseases, it was not until 1968 that an association between polyamines and cancer was established. Russell *et al.* showed that neoplastic transformation was accompanied by an increase in ODC enzyme activity (*13*). A similar association was later shown by Andersson *et al.* wherein they saw that carcinogenesis was accompanied by an increase in polyamine concentrations (*14*). The fact that intracellular polyamine content homeostasis is lost in cancer development, is evidenced from the fact that there is often an upregulation of polyamine biosynthesis enzymes with a concurrent decrease in polyamine catabolism. ODC, the rate limiting enzyme in polyamine biosynthesis is induced by cancer promoters like TPA and ultraviolet light (*15*). Transfection of normal murine NIH3T3 fibroblasts with the ODC enzyme has been shown to be sufficient in inducing the transformed phenotype (*16*). Similarly, AdoMetDC has been shown to be upregulated in certain types of cancers. Polyamine catabolic genes like SSAT have been shown to be downregulated in cancers (*17*). We, and several other groups have reported that cancer development in the Min mouse model is accompanied by a decrease in the OAZ and SSAT proteins (*18*). Thus, multiple aspects of the polyamine metabolic pathway can be perturbed. In the past few years, it has become increasingly evident that cancer development is also accompanied by an increase in polyamine uptake. Recently, Nilsson *et al.* have shown that cancer cells have significantly more polyamine uptake, as compared to normal cells, in a mouse model of lymphomagenesis (*19*).

The polyamine metabolic pathway plays an especially important role in epithelial cancer progression. The WNT signaling pathway is a major pathway governing epithelial development (*20*). Its normal functioning is lost during cancer progression. The key players in WNT signaling are the adenomatous polyposis coli (APC) and β-catenin proteins. Under normal conditions, APC sequesters β-catenin in the cytoplasm and prevents its transcriptional activity (*21*). However, aberrations in the pathway leads to nuclear translocation of β-catenin and oncogenic signaling. The *APC* gene is mutated in epithelial cancers like that of the colon. β-catenin and other components of the WNT signaling pathway are often deregulated in other epithelial cancers like that of the breast and the ovary.

The Vogelstein group identified the oncogenic transcription factor, c-MYC as a target of the APC/β-catenin signaling cascade (*22*). It is often upregulated in epithelial neoplasias. Subsequently, our group showed that ODC is a transcriptional target of c-MYC protein, thereby linking polyamines to Wnt signaling (*23*). The c-MYC oncogene is a member of the family of transcription factors called E-box proteins. These proteins bind to the consensus sequence CACGTG in the promoter sequence of genes (*24*). Since the *ODC* gene has 3 such E-boxes (one 5′ of transcription start site and two E-boxes 3′ of transcription site), the c-*MYC* oncogene can lead to increases *ODC* transcription. Fultz *et al.* have also observed that introduction of a wild type *APC* in an *APC*-deficient cell line can lead to an increase in another E-box protein, the c-MYC antagonist, MAD1 (*23*). *MAD1* and c-*MYC* coordinately regulate *ODC* transcription by heterodimerising with the third E-box protein, *MAX* (*25*). The relative levels of c-MYC and MAD1 decide *ODC* transcription in the cell. Thus, under normal circumstances, wild-type *APC* suppresses *ODC* transcription by two mechanisms: downregulating its transcriptional activator, c-MYC and upregulating the repressor, MAD1. Another observation made by our lab using cDNA microarray technology was that two polyamine metabolic pathway genes, *OAZ* and *SSAT* are also induced by a wild type *APC* (Kimberley Fultz and E.W. Gerner, unpublished observation). Thus, similar to the Min mouse model where a mutated *Apc* leads to a decrease in *Oaz* and *Ssat* expression, expression of a WT *APC* in an *APC*-deficient human colon tumor cell line leads to induction of *OAZ* and *SSAT*. Thus, polyamines might be the mediators of *WNT*-initiated carcinogenesis by regulating their own biosynthesis.

Apart from *WNT*, *K-RAS* dependent mitogenic signaling is also seen in several forms of epithelial cancers like colon and pancreas to name a few (*26*). The *K-RAS* oncogene is mutated in several types of epithelial cancers. In colon cancer, it is a relatively early mutation seen in 25–40% of reported cases. Our lab has shown that an activated form of the *K-RAS* oncogene can suppress the polyamine catabolism gene, *SSAT* (*27*). The 5′-UTR of the *SSAT* gene has consensus sequences, which are binding sites for the nuclear hormone receptor PPARγ. Peroxisome proliferator activated receptor gamma or *PPAR*γ has been reported to be a putative tumor suppressor gene (*15*). We have seen that through its activation of the MAPK pathway, K-RAS can suppress PPARγ protein levels and thereby decrease *SSAT* transcription. Since PPARγ is activated by nonsteroidal anti-inflammatory drugs like aspirin, an activated *K-RAS* can stop induction of *SSAT* by repressing *PPAR*γ transcription and abrogate the effect of NSAIDs to a certain extent. Thus, polyamines may be increased in cancer by a decrease in catabolism. In addition, an activated *K-RAS* has been reported to induce the mTOR pathway (*28*). The activity of the mTOR pathway, or the mammalian target of rapamycin pathway, is often upregulated in several cancers like that of the pancreas. One of the targets of this pathway is the eIF4E protein. It is know that eIF4E can increase *ODC* expression in certain types of cancers (*29*). Thus, *K-RAS* can increase polyamine biosynthesis in an mTOR-eIF4E dependent manner.

Apart from perturbations in the polyamine metabolic pathway, increased polyamine content in cancer can itself promote tumorigenesis. By virtue of their nucleic acid-binding abilities, polyamines can influence translation by inducing the assembly of the 30S ribosomal subunit and enhancing the synthesis of Ile-tRNA (*30*). Similarly, polyamines have been shown to decrease p53 levels, while leading to a concurrent increase in the p53 ubiquitin ligase, MDM2 (*31*). p53 is a tumor suppressor, often times mutated or downregulated in early or late stages of epithelial cancers. From the polyamine metabolism point of view,

polyamines can also activate Casein Kinase II (*1*). CKII can inactivate Glycogen Synthase Kinase 3β (GSK3β) and this can lead to more transcriptional activity of β-catenin, leading to an increased c-*MYC* and *ODC* transcription. Thus, polyamines by themselves can start a positive feedback loop to increase their biosynthesis in cells with high levels of polyamines. Lastly, polyamines are required for the post-translational modification of eIF5A. eIF5A has been reported to be over-expressed in several human cancers. One could speculate that increased polyamines could lead to an increase in active eIF5A and thus lead to an increase in protein synthesis and cell growth. Thus, polyamines can play pivotal roles in tumor promotion and progression.

Apart from that, these molecules have also been reported to act as biomarkers in epithelial cancer development. The Russell group showed that cancer patients secreted significantly more amount of polyamines as compared to normal healthy individuals (*32*). They later on went on to show that urinary polyamine content is a measure of therapeutic outcome in cancer patients. Recently, Hiramatsu *et al.* have reported that diacetylated polyamines, namely N^1,N^{12}-diacetylspermine is a novel and sensitive biomarker in early and late stage epithelial malignancies (*33*).

The polyamine pathway and anti-cancer drug development

Since polyamines and cancer seem to be intricately linked, it is not surprising at all that the polyamine metabolic pathway has received much attention in cancer drug development. ODC being the rate limiting enzyme in polyamine biosynthesis was the first target in the polyamine pathway. Abdel-Monem *et al.* synthesized the first efficacious competitive inhibitor of ODC, α-methyl ornithine (*34*). However, this drug failed to generate any excitement, because like all competitive enzyme inhibitors, α-methyl ornithine stabilized ODC protein and thus prevented its degradation. The first breakthrough in drug discovery happened in 1978 when Merrell Dow Company in Strassbourg, France synthesized the first irreversible inhibitor of ODC, difluoromethyl ornithine (DFMO) (*15*). DFMO was shown to be an extremely promising agent in *in vitro* studies. It was shown to be cytostatic in several cell lines. However, its effect were somewhat abrogated *in vivo*, because it was seen that cancer cells treated with DFMO seemed to develop a compensatory increase in polyamine uptake. Thus, the effects of DFMO seemed to be enhanced using a polyamine deficient diet, in an *in vivo* rodent system of carcinogenesis (*35*). It has also been reported that there seems to be an additive or a synergistic effect when DFMO is administered with already established chemotherapeutic agenst like cyclophosphamide, in *in vivo* carcinogenesis models. Apart from DFMO, the other polyamine biosynthesis enzymes, AdoMetDC and spermidine/spermine synthase have also received considerable attention from the medicinal chemist. Most SAMDC inhibitors rapidly deplete spermidine and spermine concentrations in the cells, with a concurrent increase in putrescine levels. Although AdoMet analogues like MDL73811 ({[(*Z*)-4-amino-2-butenyl]methylamino}-5′-deoxyadenosine) showed activity against various cancer cell lines, their effects vanished in *in vivo* models. This was presumably because the accumulation of putrescine seemed to compensate for spermidine in cells. The MGBG [Methylglyoxal-bis-(guanylhydrazone)] analogues like SAM486A (4-amidinoindan-1-one-2′-amidinohydrazone) seem to show more promise in experimental tumor models in rodent systems. This may be, in part, due to the fact that SAM486A can also inhibit PAO activity. However, this has not been conclusively established. Inhibitors of spermindine synthase (AdoDATO or *S*-adenosyl-1,8-diamino-3-thiooctane) and spermine synthase (AdoDATAD or *S*-adenosyl-1,12-diamino-3-thio-9-azadodecane) have also been developed, but have not been shown to be efficacious in both *in vitro* and *in vivo* systems.

Induction of polyamine catabolism has also been a major drug target (*6*, *36*). The enzyme spermidine/spermine acetyl transferase can be induced by a variety of compounds like NSAIDS and polyamine analogues. NSAIDS like sulindac sulfone induce *SSAT* by activating PPARγ. Others like aspirin seem to induce it thorough activation of NKkB and the NFkB response elements in the promoter region of the *SSAT* gene (*37*). Though there has been a considerable corelation between NSAIDS treatment and colon cancer chemoprevention, only recently has the molecular mechanisms governing this mechanism been elucidated. The importance of the polyamine oxidase (PAO) enzyme in cancer treatment emerged from the fact that spermine cannot reverse DFMO-induced cytostasis unless it is converted into spermidine. Keeping this in mind, several PAO inhibitors were developed, amongst which MDL72527 [N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine] is the most important. This compound has been shown to be extremely potent in *in vitro* kill curve studies with cancer cells. When administered with DFMO, it has shown a lot of promise in *in vivo* carcinogenesis models (*12*, *36*).

Other aspects of the polyamine metabolism pathway like polyamine uptake and efflux have also been targeted for cancer drug development. Uptake inhibitors like ORI202 (N^1-Spermyl-L-lysinamide) have shown promise in *in vitro* studies, but up till now, *in vivo* work has not been reported. Recently, Belting *et al.* proposed the cell surface heparin sulfate proteoglycans can act as vehicles for polyamine uptake. They showed in an *in vivo* model that treatment with a competitive inhibitor of xyloside biosynthesis increased the efficacy of DFMO as an anti-cancer agent (*38*). As previously mentioned, the activity of eIF5A is dependent on cellular spermidine levels. Inhibitors of the second hydroxylation step like N^1-guanyl-1,7- heptane (GC7) have been developed (*39*). We have seen that this compound can induce apoptosis in colon cancer cells (April Childs and E.W. Gerner, unpublished work).

Thus far, single enzyme inhibitors of the polyamine metabolism pathway have been discussed. When talking about the polyamine metabolic pathway as a target for cancer drug development, the polyamine analogues need a special mention. Polyamine analogues have pleiotropic effects on cancer cells. They can deplete cellular polyamines either by upregulating catabolism, decreasing biosynthesis by negative feedback inhibition or by competing with exogenous polyamines for uptake (*40*). They can also bind to intracellular polyamine binding sites and be "non-functional." Pharmacologically, these analogues are-classified as polyamine mimetics and polyamine antimetabolites. Mimetics like the SLIL compounds (developed

by SLIL Biomedical Corporation, Wisconsin, U.S.A.) inhibit cell growth by acting like endogenous polyamines. Antimetabolites like BENSpm [bis(ethyl)norspermine] and CHENSpm [N^1-cycloheptylmethyl-N^{11}-ethylnorspermine] decrease intracellular polyamine levels by "superinducing" the polyamine catabolism enzyme, *SSAT* (*40*).

Polyamines and cancer prevention and chemotherapy

As previously described, the polaymine metabolic pathway has been extensively studied and most of the inhibitors described above have been validated in *in vitro* and *in vivo* model systems. However, some of these drugs have failed to translate into clinical efficacy when used in cancer patients. DFMO was considered as the "magic" drug. However, clinical trials with DFMO have been disappointing. There is severe toxicity (abdominal pain, emesis, leukopenia) with doses greater than 3 gms/m^2/day (*41*). Though there was some response in Phase I toxicity studies and uncontrolled Phase II efficacy studies, DFMO could not be established as a chemotherapeutic agent in controlled clinical trials. Also, its dose-limiting toxicity was a major problem. Inspite of a lack of substantial evidence for DFMO as an anti-cancer agent, its application in adjuvant cancer drug therapy has been considerably explored. A combination therapy of DFMO with established chemotherapeutic regimens have shown promise. SAM486A is the only AdoMetDC inhibitor which has been extended to clinical trials. At the doses administered, SAM486A did not show significant dose-limiting toxicities. However, no objective remission of tumors was seen. Similarly to DFMO, combination therapies of this compound with other chemotherapeutic agents is being carried out. Polyamine catabolism inducing drugs like the analogues have also been taken to clinical trials. DEHSpm was tried out in patients with advanced solid tumors. Table 1 enlists some of the clinical trials incorporating inhibitors of the polyamine metabolic pathway.

At this stage, it is necessary to state that the polyamine pools and metabolic flux are governed by several rate constants, defining different aspects of the pathway, in a tissue-specific manner. Figure 1 illustrates some of the rates governing intracellular pools. Thus, single enzyme inhibitors may not prove to be efficacious in *in vivo* models and in human clinical trials, because their effects may depend on tissue-specific reaction rates dictating polyamine homeostasis. In order to make the therapeutic regimens more efficacious, multiple features of this pathway may need to be targeted, using combination therapy.

Though the polyamine metabolic pathway inhibitors have not been promising in clinical trials in cancer patients with established disease, research in the past few years have shown that these agents might be useful in cancer chemoprevention. Sporn defines cancer chemoprevention as "the use of natural, synthetic or biologic chemical agents to reverse, suppress, or prevent carcinogenic progression" (*42*). The concept of chemoprevention is based on the multistep and multifocal field carcinogenesis paradigm. This, simply put, implies that arresting or reversing transformation at any stage of cancer development could siginificantly decrease the risk of the disease progressing into full-blown cancer (*43*). Colorectal cancer chemoprevention can be used as a model to study cancer chemoprevention. It is based on the multi-step process of carcinogenesis, wherein cancer develops from normal epithelium through the accumulation of a series of genetic mutations. The cancer progression from normal epithelium to metastatic cancer can be divided into distinct stages (*32*, *44–46*). Though this sequence of events may not be essentially linear, this model helps us understand the interaction of various environmental and genetic risk factors in the development of colorectal cancer. Lastly, the role of the polyamine metabolic pathway in this multi-step process is understood to a large extent. It has been shown that inactivation of the wild type *APC* gene leads to an increased polyamine content because of increased *ODC* transcription. A mutation in the *K-RAS* oncogene further substantiates this effect by downregulating polyamine catabolism. A combination chemopreventative intervention using a biosynthesis inhibitor like DFMO, and an agent, which induces catabolism, should, in theory, be able to stop the disease from progressing beyond the adenoma phase. This rationale has provided the basis of two ongoing chemoprevention studies, using the NSAID sulindac and the selective COX-2 inhibitor,

Table 1. **The following table lists ongoing clinical trials in cancer chemotherapy and chemoprevention, using inhibitors of the polyamine metabolic pathway.**

Inhibitor	Mode of action	Status of clinical trial	Reference
DFMO (Chemotherapy)	ODC inhibitor	Low efficacy seen in epithelial neoplasias like colon, cervical and breast neoplasias	(*15*)
DFMO + PCV [procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, vincristine] (Chemotherapy)	ODC inhibitor + conventional nitrosourea based chemotherapeutic regimen	Survival advantage to patients receiving both, as against PCV alone, in brain tumor patients.	(*49*)
SAM486A (Chemotherapy)	SAMDC inhibitor	No significant therapeutic potential in patients with metastatic melanoma	(*50*)
DEHSpm (N^1,N^{14}-diethylhomospermine) (Chemotherapy)	Polyamine analogue capable of inducing SSAT	Further study stopped due to significant hepato- and neurotoxicity	(*51*)
DFMO + Sulindac (Chemoprevention)	ODC inhibitor + SSAT inducer	Adenoma regression (in patients with prior sporadic adenomas)	(*15*)
DFMO + Celecoxib (Chemoprevention)	ODC inhibitor + SSAT inducer	Adenoma regression (in patients with FAP)	(*15*)

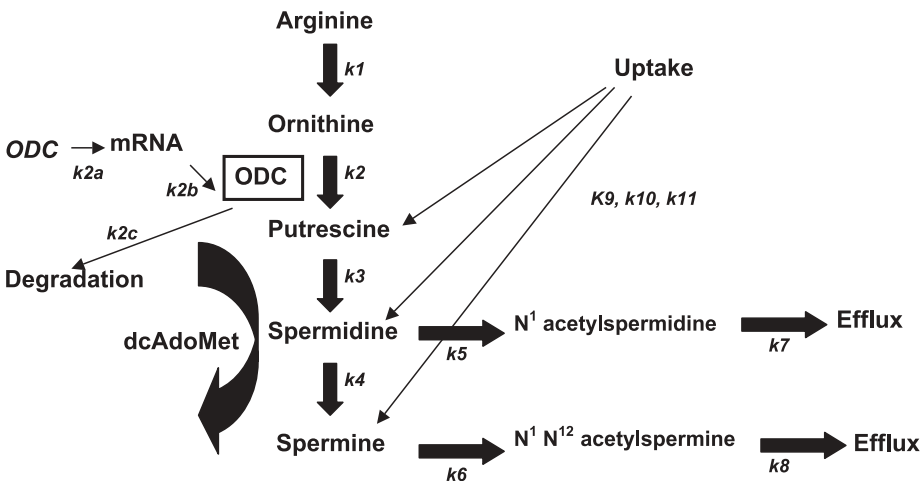

Fig. 1. **Intracellular polyamine pools and metabolic flux are controlled by different rate constants, regulating different reactions of the pathway, in a tissue-specific manner.**

celecoxib. As previously mentioned, several NSAIDS like sulindac sulfone and aspirin can lead to an increase in *SSAT* enzyme transcription by activating the nuclear horome receptor, PPARγ. One such randomized, placebo-controlled clinical trial is currently being conducted by our lab, in collaboration with Frank Meyskens at the University of California, Irvine. The trial involves administration of a combination therapy of DFMO and sulindac for colon polyp prevention. Though the trial is still ongoing (accrual 75% complete), the evidence gathered thus far shows strong evidence of activity. Another trial involving celecoxib and DFMO in colon polyp prevention is also under way at the MD Anderson Cancer Center, Texas (*15*). Apart from catabolism, another attractive target in the polyamine metabolic pathway is polyamine uptake. Several groups have shown that neoplastic transformation is accompanied by an increase in polyamine uptake. The *in vivo* effects of DFMO can be abrogated because of the development of compensatory uptake mechanisms (35). Recent data from our lab has implicated various signaling pathways like the RAS pathway in regulating polyamine uptake by colon cancer cells. Thus, a combination therapeutic approach using a combination of DFMO and a *RAS* inhibitor might prove efficacious because of a two pronged approach: decreasing biosynthesis and increasing catabolism, and by inhibiting the compensatory uptake of polyamines. Interestingly, the p53 tumor suppressor does not seem to affect polyamine levels in colorectal cancer (*47*).

ODC is also seen to be upregulated in intra-epithelial neoplasias, non-invasive precursors of epithelial cancers. Thus, it is not surprising that DFMO is also being explored as a potential chemopreventative agent in clinical trials of other epithelial cancers like non-melanoma cutaneous cancers and cervical cancer (for a recent review on the status of these clinical trials, refer to Ref. *15*).

Recent developments in polyamine-related intervention strategies in colorectal and prostate cancer

Development in molecular biology techniques has enabled us to understand the process of carcinogenesis in a mechanistic way. Difference in cancer risk because of genetic variability in a population is a well-known fact. Actual correlation between risk and variability has been emerging over the past few years. One aspect of

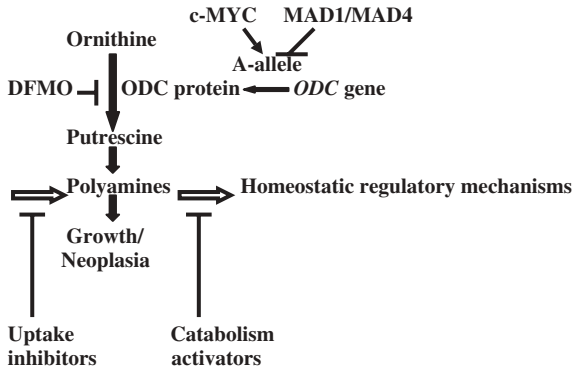

Fig. 2. **Proposed role for ODC +316 SNP in polyamine biosynthesis and response to ODC inhibitors.**

genetic variability are single nucleotide polymorphisms or SNPs. These are single base pair changes in a DNA sequence of genes, which affect the phenotype of the individual. O'Brien *et al.* reported a SNP in the *ODC* gene, between E-boxes 2 and 3 at position +316 from the transcription start site (see Fig. 2) (*48*). Martinez *et al.* have assessed the role of this genetic variability on colorectal cancer risk, in a human colon polyp prevention study (*24*). The major allele of the SNP is A and the minor is G. 55% of the participants in this prevention trial were homozygous G at this locus, 35 were heterozygous G/A. The remaining 10% of the patient group were homozygous A. All the patients had had a previous colon polyp. The chemoprevention trial was principally designed to evaluated the influence of this polymorphism on aspirin use and polyp recurrence risk. The results of the colon polyp prevention study were intriguing. It was found that patients homozygous for A at this locus showed reduced risk in colon polyp recurrence. What was even more interesting was that amongst those who took aspirin, homozygosity for the A allele resulted in an even more significant decrease in polyp recurrence risk as compared to the placebo treated groups. The mechanism of this observation was elucidated in the cell culture system using colon cancer cells. As shown in Fig. 2, it was found that the c-MYC antagonist, MAD1 selectively repressed *ODC* transcription in an A-specific manner. Aspirin however did not affect A-specific *ODC* transcription. The effect of aspirin on

decrease in adenoma recurrence was attributed to the activation of polyamine catabolism through the induction of *SSAT*.

A prediction of the above mechanism is that this A-specific repression of *ODC* should be reflected in the tissue polyamine contents. Secondly, it would also determine a tissue's response to DFMO. To evaluate the biological significance of the *ODC* SNP, we have determined its relationship to tissue polyamine content and response to DFMO in patients who are undergoing colorectal and prostate chemoprevention trials (E.W. Gerner *et al.*, manuscript submitted for publication). We found that polyamine content was dependent on the *ODC* genotype, in normal prostate tissue, but not in normal colorectal tissue, obtained from participants in cancer prevention trials. Our findings suggest that *ODC* transcription is a major rate limiting step in determining prostate tissue polyamine content. However, other processes, in addition to *ODC* transcription, appear to be rate limiting for polyamine pool size regulation in the colon.

Conclusion

Polyamine metabolic pathway genes are downstream mediators of oncogenes and tumor suppressor genes in epithelial carcinogenesis. Thus, there is strong rationale for the development of cancer therapeutic agents, targeting the polyamine metabolic pathway. However, polyamine pool sizes and flux are regulated by a number of processes, in a cell- and tissue-specific manner. Effective chemotherapeutic and chemopreventative interventions, targeting this pathway, will require a combinatorial approach directed towards multiple features of this pathway.

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