Amino Acids (2007) 33: 213–223
DOI 10.1007/s00726-007-0531-2
Printed in The Netherlands

Amino Acids

Regulation of ornithine decarboxylase during oncogenic transformation: mechanisms and therapeutic potential

Review Article

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Received October 15, 2006
Accepted February 1, 2007
Published online April 19, 2007;

Summary. The activity of ornithine decarboxylase (ODC[1]), the first enzyme in polyamine biosynthesis, is induced during carcinogenesis by a variety of oncogenic stimuli. Intracellular levels of ODC and the polyamines are tightly controlled during normal cell growth, and regulation occurs at the levels of transcription, translation and protein degradation. Several known proto-oncogenic pathways appear to control ODC transcription and translation, and dysregulation of pathways downstream of *ras* and *myc* result in the constitutive elevation of ODC activity that occurs with oncogenesis. Inhibition of ODC activity reverts the transformation of cells in vitro and reduces tumor growth in several animal models, suggesting high levels of ODC are necessary for the maintenance of the transformed phenotype. The ODC irreversible inactivator DFMO has proven to be not only a valuable tool in the study of ODC in cancer, but also shows promise as a chemopreventive and chemotherapeutic agent in certain types of malignancies.

Keywords: Ornithine decarboxylase – DFMO – Oncogenic transformation – Polyamines

Abbreviations: AdoMetDC, S-adenosylmethionine decarboxylase; AZ, antizyme; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; DFMO, α-difluoromethylornithine; DMBA, 7,12-dimethylbenz[a]anthracene; eIF4E, eukaryotic initiation factor 4E; ERK, extracellular-signal-regulated kinase; 5-FU, 5-fluorouracil; IFN, interferon; IRES, internal ribosome entry site; K14, keratin 14; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; NMBA, N-nitrosomethylbenzylamine; ODC, ornithine decarboxylase; OH-BBN, N-butyl-N(4-hydroxybutyl)nitrosamine; SSAT, spermidine/spermine N^1-acetyltransferase; TPA, 12-O-tetradecanoylphorbol-13-acetate

Introduction

The polyamines putrescine, spermidine and spermine are essential for normal cell growth and differentiation, and depletion of polyamines inhibits the growth of neoplastic cells both in vitro and in animal models. Consequently, the polyamine biosynthetic pathway may provide an important target for the development of agents that prevent carcinogenesis and tumor growth. The polyamine-metabolizing enzymes are activated by a variety of growth factors, carcinogens, viruses and oncogenes. Given the absolute requirement of polyamines for cell growth and the potentially oncogenic consequences of their overproduction, it is not surprising that the polyamine-metabolizing enzymes and polyamine levels are subjected to extensive regulation. Alterations in enzyme activities can occur very quickly, and are usually the result of changes in the absolute amount of enzyme protein. Regulation can occur at the levels of transcription, translation and protein degradation. Ornithine decarboxylase (ODC) is generally thought to be the rate-limiting enzyme in polyamine biosynthesis, catalyzing the formation of putrescine from ornithine. In addition to ODC, regulation of S-adenosylmethionine decarboxylase (AdoMetDC) and spermidine/spermine N^1-acetyltransferase (SSAT) have also been studied quite extensively (reviewed in Shantz and Pegg, 1999). Although the regulation of these three enzymes is quite complex and interdependent, this review concentrates on the regulation of ODC and its relationship to neoplastic transformation. We also review the information regarding the ODC suicide inactivator α-difluoromethylornithine (DFMO) in chemoprevention and chemotherapy of various human malignancies.

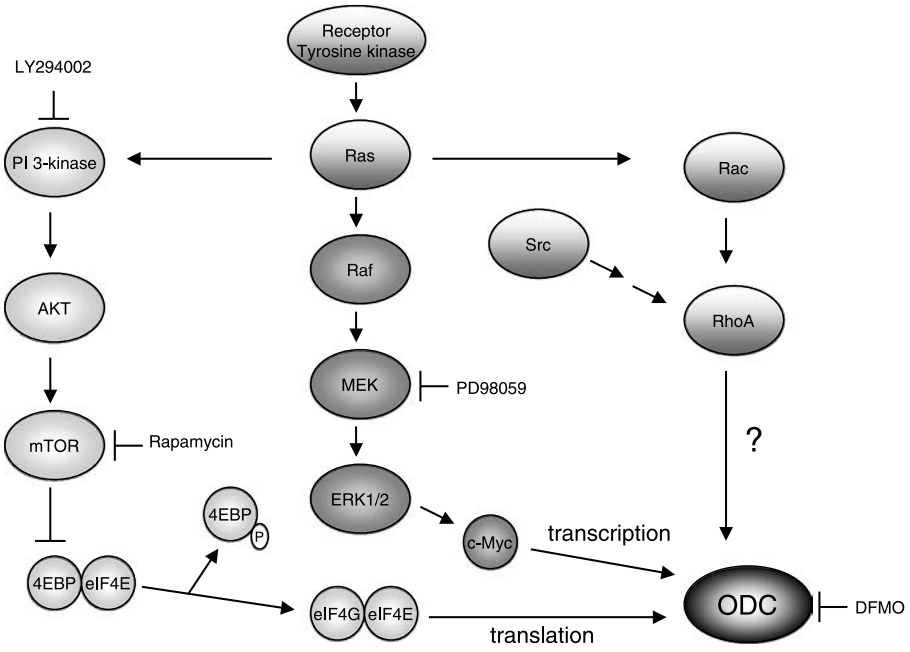


Fig. 1. Oncogenic pathways known to regulate ODC expression. Pathways involved in ODC translation are represented in light gray, while those involved in ODC transcriptional regulation are in dark gray. Other pathways for which the mechanism of regulation is currently unclear are also presented. Inhibitors of signaling intermediates and of ODC are indicated

Oncogenic activation of ODC in vitro

Regulation of ODC synthesis in transformed cells

Like most proteins important for cell growth, intracellular levels of ODC are controlled very tightly, and the regulation of ODC synthesis is extremely complex. Numerous studies have documented ODC regulation at the levels of transcription, translation and protein degradation (reviewed in Pegg, 2006; Shantz and Pegg, 1999). Likewise, multiple means of ODC dysregulation can occur in response to a variety of oncogenic stimuli. Figure 1 illustrates the major oncogenic pathways that appear to be involved in regulation of ODC transcription and translation. For example, ODC is a transcriptional target of c-*myc* that can mediate both Myc-induced apoptosis (Packham and Cleveland, 1994) and Myc-induced lymphomagenesis (Nilsson et al., 2005). Transformation by activated *ras* also appears to be tightly coupled to ODC gene expression and polyamine accumulation (Hölttä et al., 1988). ODC transcription is induced in response to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), and the importance of high ODC activity as a mediator of tumor promotion has been studied extensively, as discussed below. However, a TPA-responsive element has not been positively identified in the ODC promoter, and it has been suggested that regulation of ODC by TPA is cell-type specific (reviewed in Shantz and Pegg, 1999).

In addition to transcriptional up-regulation, there have also been reports of alternative splicing of ODC mRNA in response to transformation, leading to increased ODC synthesis (Pyronnet et al., 1998), and production of a truncated stabilized ODC protein in human hepatoma tissue (Tamori et al., 1995). A finding that links ODC directly to human cancer has been a single nucleotide polymorphism in intron 1 of the ODC gene, which results in increased ODC expression in response to Myc (Guo et al., 2000), and was identified as a genetic marker for colon cancer risk (Martinez et al., 2003).

Cap-dependent translational regulation of ODC has been well-established, and ODC activity is induced in cells that overexpress the translation initiation factor eIF4E (4E-P2 cells) (Lazaris-Karatzas et al., 1992). These cells exhibit characteristics of cellular transformation that are mediated at least in part by increased translation initiation of ODC (Rousseau et al., 1996; Shantz and Pegg, 1994). Translational regulation of ODC is unique, as it belongs to a special class of RNAs that possess very long and highly structured 5′UTRs that, in general hinder efficient translation (Shantz and Pegg, 1994). However, conditions that increase the presence of translation initiation factors such as eIF4E allow efficient translation of ODC. This may be the case in transformed cells, which generally contain higher polyamine levels compared to normal cells, yet also maintain high levels of ODC activity. Experiments in

AR4-2J pancreatic tumor cells, which show increased phosphorylation of the eIF4E regulatory protein 4E-BP1 and high levels of eIF4E compared to normal cells, also exhibit increased translational initiation of ODC mRNA (Pyronnet et al., 1998). Additional evidence that the intracellular availability of active eIF4E correlates with ODC translation has been provided in studies using IEC-6 intestinal epithelial cells, where it has been shown that Rapamycin, which blocks phosphorylation of 4E-BP1, inhibits the induction of ODC in response to serum (Seidel and Ragan, 1997).

While translation via the cap is the predominant mode of ODC translation, under specific conditions, such as the G2-M phase of the cell cycle or during apoptosis, ODC can be translated in a cap-independent manner (Pyronnet et al., 2000). Cap-independent translation is well documented for viral RNAs that lack a cap structure and are translated efficiently by recruiting ribosomes directly onto regions within their 5′UTR termed Internal Ribosome Entry Sites (IRES) (Stoneley and Willis, 2004). IRES-mediated translation has also been reported for other cellular RNAs important for cell growth, including c-myc and cyclin D1 (Stoneley and Willis, 2004). ODC exhibits a biphasic induction of activity, once at the G1-S boundary and again during G2-M (Fredlund et al., 1995). Previous studies showed that ODC activity is increased during G2-M in spite of an overall inhibition of global protein synthesis, and ODC activity during this phase was not responsive to the effects of Rapamycin, an inhibitor of translation initiation (Pyronnet et al., 2000). These results suggest that ODC activity during G2-M is regulated in a cap-independent manner. This mode of ODC translation may help to maintain high ODC activity and polyamines necessary for the rapid growth of transformed cells.

Our studies have concentrated on understanding the mechanism of ODC regulation by Ras effector pathways, and have studied in detail the mechanism by which these pathways control intracellular ODC levels. Ras is an essential component of several receptor-mediated signal transduction pathways crucial to normal cell growth and differentiation, and constitutively active Ras point mutations have been implicated in at least 20% of all human cancers (reviewed in Downward, 2003). ODC activity is dramatically induced in response to *ras* activation (Hölttä et al., 1988; Shantz and Pegg, 1998). Using Ras partial-loss-of-function mutants and inhibitors of specific Ras effectors (see Fig. 1), we have shown activation of both ODC transcription and translation in NIH/3T3 cells transformed by *ras* (Shantz, 2004). In addition, the data suggest that at least two pathways downstream of Ras must be activated to produce high levels of ODC activity, with the Raf/MEK/ERK cascade mediating ODC transcription and the PI 3-kinase cascade mediating ODC translation. Activation of these pathways thus increases levels of both ODC RNA and protein, resulting in the dramatic upregulation in ODC activity that accompanies Ras transformation.

In vitro ODC inhibition studies

High levels of ODC can transform NIH/3T3 cells (Auvinen et al., 1992), and induction of ODC activity and changes in polyamine levels have been demonstrated in a variety of experimental systems designed to study neoplastic growth. Because polyamines play essential roles in DNA and RNA function, it would be expected that inhibition of ODC would inhibit tumor growth, and possibly tumor cell migration. Indeed, early studies showed that inactivation of ODC using DFMO significantly reduced proliferation of adenocarcinoma, squamous cell, and leukemia cell lines (Prakash et al., 1980). In addition, DFMO not only inhibits proliferation, but also reverts the transformed phenotype of NIH/3T3 cells overexpressing eIF4E (Shantz and Pegg, 1994). Reduced proliferation, however, does not always correlate with loss of cancer cells. For instance, human adenocarcinoma, squamous cell carcinoma, and large cell undifferentiated carcinoma cell lines showed no cell loss over treatment periods of up to 8 weeks despite a cessation of cell proliferation during DFMO treatment (Luk et al., 1982). Similarly, in a clonogenic assay, the inhibitory effects of DFMO against human pancreatic tumor cell lines were predominantly cytostatic and reversible by addition of the ODC reaction product putrescine (Chang et al., 1984). More recent studies have shown that blocking ODC activity with DFMO causes G1 arrest in N-Myc-amplified human neuroblastoma cells (Wallick et al., 2005), IEC-6 rat intestinal epithelial cells (Li et al., 1999), and human melanoma cells (Kramer et al., 2001).

Combination therapies in vitro

Enhanced growth inhibitory activity has been observed in vitro with DFMO in combination with other agents. DFMO following cisplatin administration markedly enhanced the cisplatin-induced inhibition of colony formation in hamster pancreatic adenocarcinoma cell lines (WD PaCa and PD PaCa) (Ratko et al., 1990). Also in clonogenic assays, a DFMO-cisplatin combination was schedule-dependent against human pancreatic cell lines (PANC-1 and COLO 357) with antagonism seen when DFMO was ad-

ministered prior to cisplatin and synergism when administered after cisplatin in the PANC-1 and WD PaCa cell lines (Chang et al., 1987).

Potentiation of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) activity in vitro was observed in a SF-126 human malignant glioma cell line treated with 1 mM DFMO for 72 h (Hunter et al., 1990). This was not seen in U251 malignant glioma cells, even though 1 mM DFMO depleted intracellular putrescine and spermidine concentrations. In this study cisplatin cytotoxicity was decreased, causing a 38% decrease in the number of DNA interstrand crosslinks formed. In contrast, interstrand crosslinks in BCNU-treated cells were not affected by DFMO pretreatment. These results suggested that different mechanisms of action are involved in the modification of BCNU and cisplatin cytotoxicity by DFMO.

In vitro growth of human cell lines derived from renal carcinoma cells (KO-RCC-1), choriocarcinoma cells (Bewo), and urinary bladder carcinoma cells (HT-1197) was evaluated following exposure to DFMO in combination with alpha-, beta-, or gamma- interferon (IFN) (Gohji et al., 1986). The combination of DFMO and gamma-IFN was more inhibitory to cellular growth than DFMO in combination with either alpha- or beta- IFN. DFMO, together with gamma-IFN, synergistically inhibited KO-RCC-1 cell growth in monolayer culture and in soft agar. The other two cell lines were less susceptible to treatment. The DFMO/gamma-IFN combination also inhibited clonal growth in 10 of 11 primary renal cell carcinomas grown in soft agar (Gohji et al., 1986).

The colony forming ability of five human melanoma cell lines was reduced by 90% over any reduction by a single agent when DFMO was combined with dexamethasone, retinoic acid, and alpha-IFN (Bregman and Meyskens, 1986). Because DFMO is capable of arresting specific types of tumor cells in S phase, it was tested alone and in combination with 5-fluorouracil (5-FU) to evaluate its effectiveness against a human colon adenocarcinoma cell line, Colo 205. Both DFMO and 5-FU inhibited Colo 205 cell proliferation, and the antiproliferative effect was additive when these two agents were combined (Kingsnorth et al., 1983).

DFMO has also been evaluated in combination with inhibitors of other polyamine-metabolizing enzymes. Several studies have used DFMO in combination with inhibitors of AdoMetDC, with varying results. An additive effect was observed in human melanoma MALME-3M cells treated with DFMO and MDL-73811 (also called AbeAdo), an irreversible inactivator of AdoMetDC, in which cells arrested in G1 in the presence of both inhibitors, but not in the presence of either inhibitor alone (Kramer et al., 2001). Treatment of both LAN-1 and NMB-7 human neuroblastoma cells with DFMO in combination with the MGBG analogue SAM486A, a competitive inhibitor of AdoMetDC, resulted in rapid G1 arrest that was sustained even after removal of the inhibitors (Wallick et al., 2005). When MDA-MB-435 human breast cancer cells were implanted into mammary fat pads of nude mice, DFMO treatment alone had a dramatic effect on tumor growth, local invasion into muscle, and pulmonary metastasis. However, inclusion of SAM486A along with DFMO failed to have any additional effect (Manni et al., 2005). These studies reflect both cell type-specific differences in response to polyamine depletion, and differences in manipulating tissue polyamines using in vivo compared to in vitro models.

Regulation of ODC in vivo

Animal models

The function of ODC in carcinogenesis in vivo has been assessed against a large spectrum of neoplastic rodent and human xenograft tumor models. The antitumor action of ODC inhibition using DFMO has been demonstrated in models using IP injection of L1210 cells, Lewis lung carcinoma injected into muscle, and subcutaneous injection of B16 murine melanoma, EMT6 sarcoma, M3 murine adenocarcinoma, and Caco-2 colon carcinoma cells overexpressing Ki-ras (Bartholeyns, 1983; Ignatenko et al., 2004; Klein et al., 1985; Prakash et al., 1980; Sunkara and Rosenberger, 1987). In some cases metastases were also reduced (Bartholeyns, 1983; Klein et al., 1985; Sunkara and Rosenberger, 1987). In addition, DFMO treatment was shown to markedly inhibit tumor growth and increase mean animal survival of small cell lung carcinoma in an athymic mouse model (Luk et al., 1982) and intracerebral (IC) high-grade gliomas (Moulinoux et al., 1991). Decreased metastasis of hormone-independent breast cancer to lung and bone has also been demonstrated in the presence of DFMO (Richert et al., 2005).

The chemopreventive effects of DFMO (given as a dietary supplement) were assessed in three rodent models of human epithelial cancer (Ratko et al., 1990). DFMO provided significant protection against 7,12-dimethylbenz[a]-anthracene (DMBA)-induced mammary carcinogenesis in rats. In mice, the incidence of N-butyl-N(4-hydroxybutyl)-nitrosamine (OH-BBN)-induced bladder cancer was reduced by 54–62% versus the control group when DFMO was given prior to the administration of the first dose of

OH-BBN. Furthermore, in OH-BBN-treated mice consuming 4 g/kg DFMO in the diet, the lesions that did occur tended to be less invasive than those observed in mice receiving either DFMO at 2 g/kg or the basal diet alone. In hamsters, continuous treatment with DFMO reduced the incidence and size of tracheal carcinomas occurring in animals exposed to the carcinogen methylnitrosourea.

Combination therapies in vivo

Synergistic or additive effects on tumor growth have been observed in various animal tumor models with combinations of DFMO and other agents. The effect of DFMO in combination with vindesine or doxorubicin was compared with single-agent therapy in three different animal tumor models (Bartholeyns and Koch-Weser, 1981). Treatment with DFMO and doxorubicin or vindesine increased the mean survival time of mice inoculated with L1210 leukemia cells. Growth of hepatoma tissue inoculated into Buffalo rats was completely arrested with treatment of DFMO in combination with doxorubicin or vindesine. In combination with doxorubicin or vindesine, DFMO treatment resulted in enhanced inhibition of tumor growth in mice inoculated SC with solid EMT6 tumors (Bartholeyns and Koch-Weser, 1981). DFMO also potentiated the antitumor therapeutic activity of BCNU in animals with transplanted T9 gliosarcoma. In this combined regimen, DFMO enhanced the antitumor activity and increased the lifespan of animals receiving IP or intra-arterial BCNU (Cohen et al., 1986).

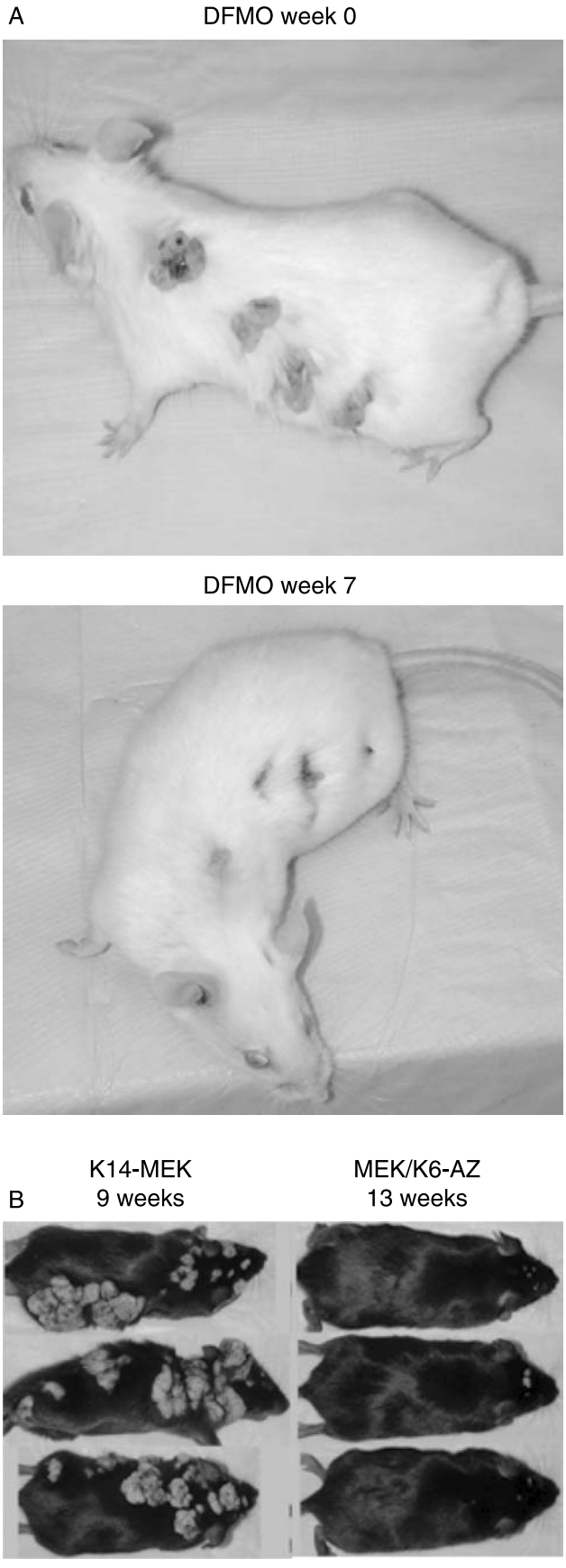
Total or near total tumor suppression was observed in mice inoculated with B16 murine melanoma cells and treated with DFMO in combination with alpha-IFN (Sunkara et al., 1983). Tilorone and polyriboinosinic: polyribocytidilic acid (poly IC), both interferon inducers, and murine alpha-, beta-, and gamma-IFN enhanced the antitumor activity of DFMO against B16 melanoma and Lewis lung carcinoma in mice (Sunkara et al., 1989).

DFMO was used along with other chemotherapeutic agents in vivo against Dunning R3327 transplantable rat prostatic adenocarcinoma to investigate whether lower dose combination therapy could enhance the antiproliferative activities of these agents while reducing their toxicity (Carvalho et al., 1989). Animals were given 1.5% DFMO in their drinking water (average daily dose = 262 mg/animal), a dose that when used alone did not significantly reduce their implanted tumors. Cyclophosphamide, cisplatin, or 5-FU was given at varying dose levels IP once weekly after tumors became palpable (day 7 after transplant). When single-agent cyclophosphamide, cisplatin, or 5-FU were given, responses were varied, but in all cases the lowest dose alone was not effective in significantly reducing tumor growth. However, all three agents were effective at the lower doses when combined with DFMO. Against IC murine glioma 26 and rat 9L gliosarcoma tumors, pretreatment with oral DFMO in the drinking water before IP administration of BCNU potentiated the effect of BCNU without increasing toxicity, while administration after BCNU was not effective (Marton et al., 1981).

Transgenic models of skin carcinogenesis

The use of transgenic animals provides a unique system for the study of the events and gene interactions that occur during tumor development. One of the most extensively studied models of carcinogenesis has been the multistep mouse skin tumorigenesis protocol, in which a single application of the initiator carcinogen DMBA is followed by promotion with the phorbol ester TPA. The genetic alterations that occur in response to this two-stage initiation/promotion protocol have been studied extensively in recent years (reviewed in Perez-Losada and Balmain, 2003), making this an ideal model in which to study the effects of transgenic overexpression or knockdown of genes of interest. The vast majority of skin tumors arising in this model possess an A-T transversion at codon 61 of Ha-*ras*, resulting in a constitutively activated Ras protein that is thought to be the result of a direct interaction between Ha-*ras* and the initiating carcinogen. Thus, activation of *ras* is an essential step in tumor initiation in this model, and several other genetic pathways have been linked to the initiation, promotion and progression stages of tumor development. These include cyclin D1 overexpression, loss of p53 expression and downregulation of E-cadherin (Perez-Losada and Balmain, 2003).

Induction of ODC is known to occur in response to TPA treatment in the DMBA/TPA model, and ODC is constitutively elevated in the resulting skin tumors (O'Brien, 1976). Transgenic mice overexpressing ODC in hair follicle keratinocytes using keratin promotors (K6-ODC mice and K5-ODC mice) were shown to be much more sensitive than littermate controls to DMBA-induced carcinogenesis, and did not require treatment with a tumor promoter to develop tumors, suggesting that ODC overexpression is a sufficient promoting stimulus in this model (Peralta Soler et al., 1998). Double transgenic mice targeting ODC overexpression to the hair follicles in conjunction with an activated Ras protein expressed via the ζ-globin promoter (ODC/Ras mice) develop spontaneous skin carcinomas without the need for initiation or promo-



tion (Lan et al., 2000), showing that Ras activation can cooperate with high ODC activity in tumor development.

Using mice that overexpress a constitutively active mutant of MEK in the skin (K14-MEK mice), our studies have implicated the Raf/MEK/ERK pathway in the regulation of ODC during skin tumorigenesis (Feith et al., 2005, 2006). K14-MEK mice exhibit moderate hyperplasia, with spontaneous skin tumor development within 5 weeks of birth without the need for chemical initiation or promotion. When K14-MEK mice were given DFMO in the drinking water from birth, there was a dramatic delay in the onset of tumor growth, and only 25% of DFMO-treated mice developed tumors by 15 weeks of age, while all untreated K14-MEK mice developed tumors by 6 weeks (Feith et al., 2005). These results confirm ODC induction as a necessary step in MEK-induced tumorigenesis. In tumor regression studies, treatment of tumor-bearing mice with DFMO dramatically reduced both tumor size and tumor number within several weeks (Fig. 2A).

To validate the results of pharmacological inhibition of ODC with DFMO, tumor formation was also analyzed in K14-MEK mice crossed with transgenic lines designed to block ODC activity. These lines use K5 or K6 promoters to overexpress antizyme (AZ), an important endogenous regulator of ODC and polyamine homeostasis, in the skin. The wild type form of ODC has an extremely short half life (20–30 min), and its degradation is mediated by AZ, which binds to the monomeric form of ODC, preventing formation of the enzymatically active homodimer, and then targets ODC for degradation. In addition to its effects on ODC, AZ also controls polyamine content by inhibiting polyamine transport into cells (reviewed in Pegg, 2006). K14-MEK mice on the ICR background were crossed with K5-AZ and K6-AZ mice on both the carcinogenesis-resistant C57BL/6 background and the sensitive DBA/2 background. Expression of AZ driven by either the K5 or K6 promoter dramatically delayed tumor incidence and reduced tumor multiplicity on both backgrounds compared to littermates expressing the MEK transgene alone. The effect was most remarkable in ICR/D2 MEK/K6-AZ mice, where double transgenic mice averaged less than one tumor per mouse for more than 8 weeks,

Fig. 2. Tumor response of K14-MEK mice to ODC inhibition with DFMO or AZ. **A** The same K14-MEK mouse at age 20 weeks (DFMO week 0) and 7 weeks after receiving 1% DFMO in the drinking water see (Feith et al., 2005). **B** The three mice with the greatest spontaneous skin tumor burden in K14-MEK mice and MEK/K6-AZ mice on the ICR/D2 background. K14-MEK mice were sacrificed at 9 weeks, while MEK/K6-AZ mice were sacrificed at 13 weeks see (Feith et al., 2006)

while K14-MEK mice averaged over 13 tumors per mouse at this age. The dramatic difference in both tumor number and tumor size between K14-MEK mice and MEK/K6-AZ mice is illustrated in Fig. 2B. In addition to our results with MEK/AZ mice, AZ suppresses tumor growth in a UV skin carcinogenesis model with mice heterozygous for the patched tumor suppressor gene (Tang et al., 2004). These results suggest that AZ can act as a tumor suppressor in several skin carcinogenesis models.

Interestingly, the tumor suppressing effects of AZ expression were not duplicated by expression of an ODC dominant-negative mutant in mouse skin (Feith et al., 2006), although expression of the same ODC mutant, which lacks the lysine 69 and cysteine 360 residues essential for enzymatic activity, reverts the transformed phenotype of cells overexpressing oncogenic H-Ras or an activated mutant of RhoA, a key regulator of actin cytoskeletal organization (Shantz and Pegg, 1998). These differing results perhaps reflect the ability of AZ to block the transport of polyamines into cells.

The experiments described above establish ODC as an important component of skin tumor promotion. However, the role of ODC in the later stages of tumorigenesis is less well-defined. Polyamine depletion using DFMO has been shown to cause regression of both papillomas and squamous cell carcinomas in several transgenic models (Feith et al., 2005; Lan et al., 2000; Peralta Soler et al., 1998; Wheeler et al., 2003), but the molecular mechanisms responsible are not known. Tumor regression in K14-MEK mice treated with DFMO was the result of both inhibition of proliferation and increased apoptosis in tumors (Feith et al., 2005). However, DFMO treatment in other skin tumor models caused tumor regression either without reducing proliferation in ODC/Ras mice (Lan et al., 2000) or without causing apoptosis in K6-ODC mice (Peralta Soler et al., 1998). A recent study using a model designed to assess tumor progression has shown that in primary keratinocytes, overexpression of a constitutively active Ras12V mutant, which activates all Ras effector pathways, did not cause invasion in tracheal xenotransplants. Keratinocytes from transgenic mice overexpressing ODC in the skin (K6-ODC mice) were also non-invasive, but acquired an invasive phenotype upon expression of a Ras12V/35S mutant, which selectively activates Raf/MEK/ERK signaling (Hayes et al., 2006). This study points to a threshold of ODC activity needed for tumor cell invasion, which cooperates with other pathways. Therefore, genetic or epigenetic changes during tumor progression may be responsible for constitutive elevation of ODC activity in skin tumors, which is necessary to maintain a malignant phenotype.

Table 1. Examples of genetically altered mouse models available for studies examining the role of ODC and other polyamine-metabolizing enzymes in cancer

Tumor type	Promoter	Gene target
Skin tumors	ζ-globin K5	ν-Ha-Ras E2F-1, Cyclin D1 c-Myc, PKCα, δ, ε
	K14	MEK1, HPV-16 PKCα, δ, ε, Bcl-X_L
Lymphoid malignancies	Eμ	c-Myc, N-Myc ν-Abl, H-Ras, Bcl-2
Lung cancer	SPC	T-antigen c-Myc + EGF
Hepatocellular carcinoma	MT, albumin	TGFα, c-Myc
Breast cancer	MMTV	Neu, TGFα, Cyclin D1
Prostate cancer	probasin (TRAMP)	T-antigen
Intestinal/colon cancer	Spontaneous mutation	$APC^{Min/+}$
	Knockout	Smad3

Adapted from Herzig and Christofori (2002)

Other transgenic models

In addition to models of skin tumor development, a growing number of genetically modified mice have become available in recent years. The models noted in Table 1 provide an excellent opportunity to assess the importance of ODC and other polyamine-metabolizing enzymes in a variety of malignancies. Although many transgenic and knockout strains are available in addition to those listed in Table 1, these examples reflect tissues for which in vitro or animal data have suggested a relationship between high polyamine content and tumor development. Some of these models have been utilized in recent experiments to demonstrate the chemotherapeutic and chemopreventive effects of inhibiting the high ODC activity associated with tumor growth. In addition to those experiments discussed above, a recent report has demonstrated using Eμ-Myc transgenic mice that inhibition of ODC with DFMO delays Myc-induced lymphomagenesis (Nilsson et al., 2005). Using $APC^{Min/+}$ mice, which have a spontaneous mutation in the adenomatous polyposis coli gene and are predisposed to the development of intestinal tumors, it has been shown that DFMO treatment decreased small intestine tumor number and burden, and also increased intestinal apoptosis (Yerushalmi et al., 2006). Chemoprevention of prostate cancer in TRAMP mice has also been demonstrated using DFMO (Gupta et al., 2000). Finally, experiments using a zinc-deficient mouse model of forestomach carcinogenesis showed that either transgenic overexpression of AZ or administration of DFMO re-

versed cell proliferation and counteracted N-nitrosomethylbenzylamine (NMBA) tumor initiation by stimulating apoptosis (Fong et al., 2003).

It is important to note that, although DFMO treatment generally causes substantial reduction of both putrescine and spermidine in vitro, extensive depletion of the polyamines is not needed to produce an antitumor effect in these animal models. Our MEK/AZ mice exhibit about a 6-fold reduction in tumor putrescine levels but little change in spermidine and spermine (Feith et al., 2006), while in K14-MEK mice treated with DFMO, tumor spermidine and spermine levels actually increased slightly, with tumor putrescine decreasing 4-fold (Feith et al., 2005). Similar results were obtained in $APC^{Min/+}$ mice, where DFMO treatment reduced putrescine by about 5-fold in the small intestine, but reduced spermidine only about 2-fold, and increased spermine (Yerushalmi et al., 2006). It has also been shown the neither spermidine nor spermine levels were changed in K6-ODC mice given DFMO (Peralta Soler et al., 1998). The common factor in these models is therefore a significant decrease in putrescine. In addition, both AZ expression and DFMO treatment had very subtle effects on polyamine levels in the forestomach of mice on a zinc-deficient diet, yet caused significant reductions in NMBA-induced tumorigenesis (Fong et al., 2003). It has been shown using *Odc*(+/−) mice that a modest reduction in ODC activity can lead to a marked resistance to both skin tumor development (Guo et al., 2005) and lymphomagenesis in Eμ-Myc mice (Nilsson et al., 2005).

ODC as a drug target in human malignancies

Any discussion of ODC as a drug target for human disease revolves around DFMO, also called eflornithine. Unlike many of the tyrosine kinase inhibitors approved and under investigation today, which have multiple targets, DFMO targets only ODC. DFMO was approved by the U.S. FDA in 1990 (Ornidyl®), and by the European Community regulatory authority in 1991 for the treatment of *Trypanosoma brucei gambienses* sleeping sickness. Eflornithine hydrochloride cream, 13.9% (Vaniqa™), has been marketed in the United States since 2000 as a topical cream approved for the reduction of unwanted facial hair in women. While not approved for the treatment of cancer in humans, DFMO has been vigorously investigated for both the treatment and prevention of human malignancies.

Studies in humans followed many years of experimental studies in cell culture and rodent cancer models, as discussed above. These studies showed that ODC inhibition could slow tumor growth, reduce tumor incidence, and interact in an additive manner with some cytotoxic chemotherapeutic agents. As expected, much of the effect of DFMO can be reversed by the addition of putrescine to the media and, at times, to rodent chow when DFMO was studied in rodent tumor models. Nonetheless, even though polyamines are nearly ubiquitous in our diets, DFMO clinical trials in humans have shown antitumor activity against recurrent tumors in phase II studies and with cytotoxic drugs in upfront phase III studies. In addition, other human trials have focused on the effect of chronic daily low-dose DFMO to prevent malignancy in patients predisposed by virtue of associated morbid history or an existing precancerous lesion. In these ''prevention'' trials, preliminary studies were encouraging. Large definitive randomized studies are ongoing. In the sections that follow we will discuss the implications of both chemoprevention and chemotherapeutic studies to our understanding of ODC function in mammalian normal and cancer cells.

Chemoprevention studies and clinical trials

Based on observations obtained using animal models, clinical trials were initiated to evaluate DFMO as a chemoprevention agent for Barrett's esophagus, a premalignant lesion in the lower esophagus, which can lead to adenocarcinoma of the esophagus. The ODC level of the Barrett's lesions were significantly higher than the normal mucosa and the trial not effective (Garewal et al., 1992). DFMO has been or continues to be evaluated as a prevention strategy for many cancers, including superficial bladder cancer, cervical cancer, colorectal cancer, breast cancer, prostate cancer, and nonmelanoma skin cancer. The value of DFMO as a chemopreventive agent in these various malignancies has been the subject of several recent reviews (Gerner and Meyskens, 2004 and references therein). At this juncture we await the completion of all DFMO chemoprevention trials before concluding the whether partial ODC inhibition can be viewed as a successful cancer prevention strategy in humans.

Human cancer chemotherapy studies

When used as single therapeutic agents, polyamine inhibitors such as DFMO have shown unpredictable clinical activity against human tumors. Phase II (efficacy) studies have been conducted in non-small cell lung cancer and metastatic colon cancer (Abeloff et al., 1986). Against metastatic melanoma, DFMO alone produced disease stabilization in 33% of patients (Meyskens et al., 1986) and

also in 33% when combined with IFN-alpha (Talpaz et al., 1986). However, no survival advantage was observed (Creagan et al., 1990). Against breast cancer, no consistent benefit was seen, although an occasional patient appeared to benefit by slowed progression (O'Shaughnessy et al., 1999).

The situation with malignant gliomas differed from prior trials in that efficacy was seen in both phase II and phase III studies. DFMO was evaluated alone (Levin et al., 1987), with MGBG (Levin et al., 1987), and in a randomized study with PCV (procarbazine, CCNU, vincristine) (Levin et al., 2003). DFMO produced improvement in time to tumor progression, progression-free survival, and patient survival with only modest toxicity, thus showing promising clinical results in this patient population. The studies indicated that patients with anaplastic glioma (AG, WHO grade 3) tumors benefitted more than those with glioblastoma (GBM, WHO grade 4) tumors (Levin et al., 2003). The implications of these studies are important to consider in order to better understand ODC and polyamine synthesis as a target for cancer chemotherapy.

We have hypothesized that differences in response between the two study populations above (AG vs. GBM) may be due, in part, to differences in tumor ODC levels, because patients having tumors with relatively low levels of ODC appear to respond better to DFMO and DFMO-nitrosourea combinations. This conclusion is based on published observations showing that: 1) ODC levels are directly correlated with malignancy grade of most tumors including gliomas (Ernestus et al., 2001; Levin et al., 2004; Scalabrino and Ferioli, 1985); 2) DFMO showed less efficacy alone in patients with GBM than in patients with AG (Levin et al., 1987), who historically have lower ODC levels; and 3) for DFMO in combination with BCNU or PCV, greater efficacy (progression-free survival) was observed in AG compared to GBM patients (Levin et al., 2003). Recently, we had an opportunity to measure tumor ODC levels in patients treated with DFMO and PCV from the randomized phase III studies (Levin et al., 2003) and found that ODC levels, as measured by enzymatic activity, were inversely related to progression-free survival of these patients (Levin et al., unpublished observations). In a Cox proportional hazards model, progression-free survival was found to be inversely related to median tumor ODC activity, with an unadjusted hazard ratio for median ODC group (>3.3 nmol/30 min/μg protein vs. ≤ 3.3) of 5.8 ($p < 0.0001$); a median progression-free survival of 522 weeks for patients with AGs with median ODC activity ≤ 3.3 nmol/30 min/μg protein and 39 weeks for the 8 AG and 10 glioblastoma patients with ODC activity >3.3 nmol/30 min/μg protein. Of AG tumors in which ODC activity was evaluated, 26% had ODC levels >3.3 nmol/30 min/μg protein, indicating that it is not unique only to glioblastoma tumors. These results suggest that it may be valuable to measure tumor ODC levels prior to initiating a DFMO-based therapy in patients with high-grade gliomas. Further, patients with lower tumor ODC activities may be expected to have a more durable response to DFMO than those with higher ODC activity levels.

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