A Definitive Role of Ornithine Decarboxylase in Photocarcinogenesis

Nihal Ahmad,* Anita C. Gilliam,*
Santosh K. Katiyar,* Thomas G. O'Brien,[†] and
Hasan Mukhtar*

From the Department of Dermatology,* Case Western Reserve
University and University Hospitals of Cleveland, Cleveland,
Ohio; and The Lankenau Institute for Medical Research,[†]
Wynnewood, Philadelphia, Pennsylvania

Excessive exposure of solar ultraviolet (UV) radiation, particularly its UVB component, to human skin is the major cause for more than a million new cases of cutaneous malignancies diagnosed annually in the United States. Photocarcinogenesis, like other cancers, is a multistep process that includes initiation and promotion. A proper understanding of the molecular events occurring during the tumor promotion phase of photocarcinogenesis could lead to the development of novel approaches for the management of skin cancer. Using a transgenic mouse model (K5/ODC mice), which overexpresses the enzyme ornithine decarboxylase (ODC) in hair follicle keratinocytes, we studied the role of this gene in photocarcinogenesis. A single UVB-exposure of 180 mJ/cm² to the transgenic mice resulted in a minimal increase in bifold skin thickness and ODC activity. However, in SKH-1 hairless mice, the most common and highly sensitive model for photocarcinogenesis, and in littermate nontransgenic mice, increases in skin thickness and ODC activity were substantial. In long-term experiments, mice were exposed to 180 mJ/cm² of UVB radiation three times a week for 2 weeks (tumor-initiating dose). At 30 weeks after this treatment, in two independent experiments, 40% of the K5/ODC transgenic mice exposed to UVB were found to develop epidermal tumors. The tumors were histologically verified as benign papillomas and squamous cell carcinomas. Interestingly, 100% of the transgenic mice also developed >20 pigmented cysts/mouse, which contained keratinocyte material with increased keratinocytic melanization. Under similar UVB-exposure protocol, the nontransgenic littermates or SKH-1 hairless mice did not develop tumors or pigmented cysts for up to 50 weeks. Oral consumption of α -difluoromethylornithine, an irreversible specific inhibitor of ODC, in the drinking water (1% w/v) to the transgenic mice resulted in complete prevention of UVB-mediated tumorigenesis and a substantial decrease in the formation of pigmented cysts

(<10 per mouse). These data establish a definitive role of ODC in the promotion phase of photocarcinogenesis. (Am J Pathol 2001, 159:885–892)

Excessive exposure of skin to ultraviolet (UV) light, particularly in the middle wavelength range (UVB; 290 to 320 nm), elicits a variety of adverse effects that include skin aging, cutaneous inflammation, erythema, immunosuppression, cell death, and skin cancer.^{1–6} More than a million new cases of nonmelanoma skin cancers, which include basal cell carcinoma and squamous cell carcinoma (both derived from epidermal keratinocytes), are diagnosed annually in the United States.^{6,7} Unlike chemically induced skin carcinogenesis, UV is a complete carcinogen with both tumor-initiating as well as tumor-promoting effects.^{5,6,8} Between these two events, promotion phase is critical for cancer outcome because it leads to the clonal expansion of initiated cell population. The molecular mechanisms of UVB-mediated skin carcinogenesis, especially of tumor promotion, are poorly established because the initiation and promotion stages during the process of photocarcinogenesis are not clearly dissected. The lack of appropriate model systems is another limiting factor in this regard.

Ornithine decarboxylase (ODC) is the first and the rate-limiting enzyme in the polyamine biosynthetic pathway.^{9–11} A considerable body of information suggests that ODC plays an important role in both normal cellular proliferation, and the growth and development of tumors.^{9–12} The most convincing evidence for the role of ODC in tumorigenesis comes from studies in mouse skin using chemical carcinogenesis protocols and from studies in which diversified tumor promoters were shown to induce ODC activity and α -difluoromethylornithine (DFMO), a specific irreversible inhibitor of ODC, was shown to inhibit tumor formation.^{13–16} The sole biological function of DFMO seems to be the inhibition of ODC.^{13,17–19} Similarly, many other ODC inhibitors have

Supported by a Career Development Award from the Dermatology Foundation, USA (to N. A. and A. C. G.); the United States Public Health Service (grants RO3 AR45033 and RO3 46423 to A. C. G., RO1 CA 78809 and P30 AR 39750 to H. M., and RO3 CA-89723 to N. A.) and the Animal Experimentation Core of the Skin Diseases Research Center (core grant P30 AR 39750).

Accepted for publication May 7, 2001.

Address reprint requests to Nihal Ahmad, Ph.D., Department of Dermatology, Case Western Reserve University, 11100 Euclid Ave., Cleveland, OH 44106. E-mail: nxa3@po.cwru.edu.

been shown to possess anti-tumor-promoting effects, especially in mouse models of multistage chemical carcinogenesis of skin. Recently, transgenic mouse models overexpressing ODC in epidermal keratinocytes have been established.²⁰⁻²⁴ In one of these models, a bovine KIII (K5) promoter/regulatory region drives expression of the truncated ODC protein in basal keratinocytes of the interfollicular epidermis as well as the outer root sheath of the hair follicle.^{20,21} These mice develop skin tumors after initiation with a nontumorigenic dose of the classical tumor initiator 7,12-dimethylbenz(a)anthracene, thereby suggesting that ODC is sufficient for tumor promotion in mouse skin initiated with a chemical carcinogen.²⁰ When K5/ODC mice are crossed with Tg.Ac mice that develop skin tumors without the need of a tumor-initiating agent,^{25,26} the offspring develop skin tumors without the application of a tumor-initiating or tumor-promoting agent,²⁷ further suggesting the involvement of ODC during the promotion phase of skin carcinogenesis.

Because UVB radiation exposure to mouse skin results in induction of epidermal ODC activity, for a long time there has been the suggestion that ODC may also play a critical role in tumor-promoting effects of UVB. However, the role of ODC during UV responses is not clear. In this study, we found that limited UVB exposure (tumor-initiating dose) to K5/ODC transgenic mice was sufficient for the development of many types of skin tumors including squamous papilloma and squamous cell carcinoma. They also developed pigmented epidermal inclusion cysts and sebaceous cysts. Interestingly, under similar UVB-exposure protocol used, SKH-1 hairless or littermate nontransgenic mice did not develop tumors or cysts. To our knowledge, this is the first study showing a definitive role of ODC during photocarcinogenesis.

Materials and Methods

Animals

The B6.C3TgN(K5 ODC_{tr})26tgo mice hereafter referred to as "K5/ODC transgenic mice" used in this study were developed and bred at The Lankenau Institute for Medical Research, Wynnewood, PA. This line was produced by pronuclear microinjection of a DNA construct in which the bovine KIII (K5) promoter/regulatory region present in a cytokeratin KIII/KIV minilocus expression vector drives expression of the truncated ODC protein in basal keratinocytes of the interfollicular epidermis as well as the outer root sheath of the hair follicle.^{20,21} For tumorigenesis experiments, the transgenic mice used were backcrossed to C57BL/6J mice. The nontransgenic littermates were also obtained from the Lankenau Institute for Medical Research. Six-week-old female mice were used in this study. The SKH-1 hairless mice (females, 6 weeks old) were obtained from Charles River Laboratories (Wilmington, MA). During the entire experimental protocol, the animals were housed in the Animal Resource Facility of Case Western Reserve University, received laboratory chow diet and drinking water *ad libitum*, and were subjected to a 12-hour light/12-hour dark cycle.

UVB Exposure

In our experiments, we used short-term as well as long-term exposure protocols. The dorsal skins of animals were exposed to UV irradiation from a band of six FS-40 fluorescent lamps (National Biological Corp., Twinsburg, OH) from which UVB and UVC wavelengths, not normally present in natural solar radiation, were filtered out using Kodacel cellulose film (Eastman-Kodak, Rochester, NY). The filtered light contained mainly the radiations in the wavelength of UVB range (290 to 320 nm). The mice were anesthetized by ketamine hydrochloride injection (Parke-Davis, Morris Plains, NJ) before UVB exposure to immobilize them so that a uniform and complete UVB dose could be delivered to the dorsal skin of the mice. UVB emission was monitored with an IL-443 phototherapy radiometer (International Light, Newburyport, MA) equipped with an IL SED 240 detector fitted with a W side-angle quartz diffuser and a SC5 280 filter. The transgenic animals as well as SKH-1 mice were hairless, however, the nontransgenic littermates, which contain hair on the skin, were shaved with electric clippers and Nair depilatory lotion was applied for 3 minutes and then washed. These animals were subjected to UV exposure 24 hours after the application of Nair depilatory.

For short-term experiments, the ODC transgenic mice or the wild-type littermates were divided into two groups of eight animals each. The first group, which did not receive any treatment, served as control whereas the second group of animals was subjected to a single UVB exposure (180 mJ/cm²).

For the long-term experiments, 16 mice were divided into two groups of eight animals each. The first group of animals did not receive any treatment and served as control. In the second group, the mice were subjected to 180 mJ/cm² of UVB irradiation three times per week (on Monday, Wednesday, and Friday), for 2 consecutive weeks, with a total of six exposures representing a cumulative dose of 1080 mJ/cm². The animals were closely observed for skin tumorigenesis and the data were recorded on a weekly basis. This experiment was repeated with one additional group of animals fed with DFMO as described on the next page.

ODC Enzyme Activity

Six hours after short-term UVB exposure protocol (detailed above), the animals were sacrificed by cervical dislocation, and the skin was surgically removed and made free of connective tissue and fat. The epidermis was separated from the whole skin as reported previously²⁸ and was homogenized at 4°C in a glass-to-glass homogenizer in 10 volumes of ODC buffer [50 mmol/L Tris-HCl buffer (pH 7.5) containing 0.1 mmol/L ethylenediaminetetraacetic acid, 0.1 mmol/L dithiothreitol, 0.1 mmol/L pyridoxal-5-phosphate, 1 mmol/L 2-mercaptoethanol, and 0.1% Tween-80]. The homogenate was centrifuged at 14,000 × *g* at 4°C and the ODC enzyme activity was measured in the supernatant by measuring the release of ¹⁴CO₂ from DL-[1-¹⁴C] ornithine hydrochloride.

ride, as described previously.²⁹ Briefly, 100 μ l of the supernatant was added to 0.25 ml of the assay mixture (35 mmol/L sodium phosphate, pH 7.2, 0.2 mmol/L pyridoxal phosphate, 4 mmol/L dithiothreitol, 1 mmol/L ethylenediaminetetraacetic acid, 0.4 mmol/L L-ornithine containing 0.5 μ Ci of DL-[1-¹⁴C] ornithine hydrochloride) in a 15-ml corex centrifuge tube equipped with rubber stoppers and central well assemblies containing 0.2 ml ethanolamine and methoxyethanol in 2:1 (v/v) ratio. After the incubation at 37°C for 60 minutes, the reaction was terminated by the addition of 0.5 ml of 2 mol/L of citric acid, using a 21-gauge needle/syringe. The incubation was further continued for 1 hour after which, the central well containing the ethanolamine:methoxyethanol mixture with trapped ¹⁴CO₂, was transferred to a vial containing 10 ml of toluene-based scintillation fluid and 2 ml of ethanol. The radioactivity was measured in a Beckman LS 6000 SC liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA).

Skin Edema

To assess the effect of UVB on skin edema in ODC/K5 transgenic mice and nontransgenic littermates, the increases in bifold skin thickness and ear-punch weight were measured at 24 hours after short-term UVB exposure (180 mJ/cm²). The skin thickness was measured with a micrometer and the increase in bifold skin thickness was obtained by subtracting the values for the untreated control animals from those for the treated animals (UVB exposed). At least eight determinations were made at different sites on the dorsal skin per mouse. For increase in ear-punch weight studies, after short-term UVB exposure, punch skin biopsies from ear (4-mm diameter, four from each ear) were obtained and weighed immediately. An increase in the weight after UVB exposure was obtained by subtracting the values for the untreated controls from UVB-treated animals.

Tissue Processing and Histopathology

The epidermis from control and treated mice and tumors from tumor-bearing animals were obtained and fixed overnight in 10% zinc-buffered formalin and then transferred to 70% ethanol. Sections (4 μ m) were cut from paraffin-embedded tissue, mounted on slides, and stained with hematoxylin and eosin (H&E). The samples were examined microscopically for hyperplasia (in short-term experiments), and for tumor characteristics (in long-term experiments). Epidermal hyperplasia was determined by assessing vertical epidermal thickness and number of vertical epidermal layers. The tumors were classified as "pigmented follicular inclusion cysts," "sebaceous cyst," "squamous cell carcinoma," and "papilloma" by a dermatopathologist (ACG).

Treatment of Animals with DFMO

To evaluate the effect of DFMO, the specific inhibitor of ODC, on UVB-mediated tumorigenesis, 24 mice were

Figure 1. Effect of UVB on epidermal ODC enzyme activity in wild-type versus K5/ODC transgenic mouse skin. The animals were subjected to a single dose of UVB (180 mJ/cm²) and ODC enzyme activity was measured in the epidermis as described in Materials and Methods. *P* < 0.001, Student's *t*-test, control versus UVB-treated animals.

divided in three groups of eight mice. The first group of mice did not receive any treatment, and served as controls. The second group of animals was subjected to UVB exposure (180 mJ/cm², three times a week for 2 weeks) as detailed above. These two groups of mice also served as a repeat of the tumorigenesis experiment detailed above. The third group of animals, after the last UVB exposure, was given DFMO (1% w/v; obtained from Ilex Oncology, San Antonio, TX) in the drinking water, as the sole source of drinking fluid, for 24 weeks (30 weeks of age), which was the cessation time of the experiment.

Results and Discussion

An understanding of the molecular mechanism(s) of UVB-mediated skin carcinogenesis is far from complete. However, *in vitro* and *in vivo* studies have clearly shown the role of *p53* and *WAF1/p21* and several other genes in UV response.^{6,30,31} Studies have clearly shown that *p53* mutation is an early event in UV skin carcinogenesis and the clonal expansion of *p53*-mutated cells leads to development of skin cancer.^{4,5}

In the present study, using "K5/ODC-transgenic mice," nontransgenic littermates, and SKH-1 hairless mice, we investigated the hypothesis that ODC plays a key role during UVB-mediated responses, including the develop-

Figure 2. Effect of UVB on skin edema in wild-type versus K5/ODC transgenic mouse skin. The animals were subjected to a single dose of UVB (180 mJ/cm²) and skin edema was assessed by measuring the effect on skin thickness (A) and ear-punch weight (B) as described in Materials and Methods.

Figure 3. Effect of UVB on epidermal hyperplasia in wild-type *versus* K5/ODC transgenic mouse skin. The animals were subjected to a single dose of UVB (180 mJ/cm²) and epidermal hyperplasia was determined microscopically (at ×40 magnification) in skin sections stained with H&E, as described in Materials and Methods. Representative pictures are shown.

ment of skin cancer. It is important to mention here that these transgenic mice are known to have significant differences and abnormalities in phenotype and skin histology. A detailed account of these abnormalities and the basis for the hairless phenotype of the transgenic animals has been documented earlier in detail.³²

Increasing evidence indicates that ODC and polyamines have an important role in the regulation of cell proliferation and in the development of cancer.¹⁸ Growth induction of normal cells is known to be accompanied by a rapid transient increase in ODC activity.^{33,34} Cell transformation induced by oncogenes such as *v-src*, *neu*, and *ras* has been shown to be associated with constitutively elevated ODC activity.^{18,35–37} The up-regulation of ODC is considered essential for cell transformation. Studies have demonstrated the role of ODC in the development of chemically induced skin cancer.^{20,38–40} Studies have shown that diversified tumor promoters induce ODC activity, and DFMO, the specific irreversible inhibitor of

ODC inhibits tumor formation.^{13–16} The molecular mechanism(s) of photocarcinogenesis is poorly established because of the lack of appropriate model systems. In this study, to investigate the definitive role of ODC in skin cancer development, we used a unique a transgenic mouse model overexpressing ODC in epidermal keratinocytes. We assessed the effect of ODC overexpression on UVB exposure-mediated formation of skin edema and skin hyperplasia, which are considered as the characteristic features of tumor promotion and widely used as short-term markers relevant to skin carcinogenesis.^{41,42}

As a first step of our study, we determined the effect of short-term UVB exposure on epidermal ODC activity in the skin of K5/ODC-transgenic mice and compared it with the levels in the corresponding wild-type littermates. As shown by the data in Figure 1, the constitutive level of ODC in epidermis was found to be substantially elevated (27.9-fold) in K5/ODC-transgenic mice as compared to the nontransgenic littermates. Exposure of the skin to

Figure 4. Formation of skin lesions by limited UVB exposure in K5/ODC transgenic mice. The mice were subjected to UVB (180 mJ/cm² three times per week for 2 weeks; cumulative dose of 1080 mJ/cm²), and followed for the formation of skin lesions. Details are given in Materials and Methods. Typical representative pictures are shown.

Figure 5. Effect of limited UVB exposure on skin tumorigenesis in K5/ODC transgenic mice. The mice were subjected to UVB (180 mJ/cm² three times per week for 2 weeks; cumulative dose of 1080 mJ/cm²), and followed for the formation of skin tumors on a weekly basis. Details are given in Materials and Methods.

UVB resulted in a significant increase in ODC levels in nontransgenic littermates (3.6-fold) but only a modest increase was observed in K5/ODC-transgenic mice (1.2-fold). The lack of significant increase in ODC activity in transgenic mice by UVB is probably because of the elevated levels of this enzyme and its product, putrescine.²⁰

We also assessed the effect of short-term UVB exposure on skin edema by determining the increases in bifold skin thickness and ear-punch weights both in K5/ODC-transgenic mice and in nontransgenic littermates. As shown by the data in Figure 2, the UVB exposure was found to result in a substantial increase in bifold skin thickness as well as in ear-punch weights, although to a varied extent. It is important to mention here that studies have shown that an increase in ear weight may be associated with increased vascular permeability and that ODC is involved in the regulation of angiogenesis.^{43,44} Therefore, it may be likely that a UVB-mediated increase in ear-punch weight may have an association with increased angiogenesis. Further studies are, however, needed to firmly establish this notion. Next, we compared the effect of short-term UVB exposure on epidermal hyperplastic response by microscopic evaluation of H&E-stained tissue sections. As shown in Figure 3, UVB exposure resulted in an apparently significant increase in

epidermal hyperplasia in K5/ODC-transgenic mice as well as in the nontransgenic littermates. However, it seems that the hyperplastic response of UV was more pronounced in K5/ODC-transgenic mice than in the nontransgenic littermates.

In two independent experiments, we subjected the K5/ODC-transgenic mice, nontransgenic littermates, and SKH-1 hairless mice to limited UVB exposure (180 mJ/cm² dose for 3 days a week for 2 weeks; cumulative dose of 1080 mJ/cm²) and monitored the animals for up to 30 weeks (for K5/ODC-transgenic mice) or 50 weeks (for nontransgenic littermates and SKH-1 hairless mice). We included the SKH-1 hairless mice in this study as these represent the most accepted animal model for photocarcinogenesis and UV response studies. The dose of UVB used in this experiment is regarded as a tumor-initiating dose. As shown by the representative photographs in Figure 4 and tumor data in Figures 5 and 6, in both the experiments, 8 of 20 UVB-exposed transgenic mice developed epidermal tumors, whereas each transgenic mouse developed >20 pigmented cysts. At the 29th week the average number of tumors per mouse was 0.67 ± 0.13 whereas an average of 21.9 ± 1.6 pigmented cysts were present per mouse. The tumors were histologically verified as squamous cell carcinoma and squamous papilloma. The cysts were classified as sebaceous

Figure 6. Effect of limited UVB exposure on the formation of pigmented cysts in K5/ODC transgenic mice. The mice were subjected to UVB (180 mJ/cm² three times per week for 2 weeks; cumulative dose of 1080 mJ/cm²), and followed for the formation of pigmented cysts on a weekly basis. Details are given in Materials and Methods.

Figure 7. Formation of skin tumors and pigmented cysts by limited UVB exposure in K5/ODC transgenic mice. **A:** Squamous cell carcinoma (original magnification, $\times 40$). **B:** Papilloma (original magnification, $\times 40$). **C:** Pigmented epidermal inclusion cysts (original magnification, $\times 10$). **D:** Sebaceous cysts (original magnification, $\times 40$). The mice were exposed to limited UVB radiations (cumulative dose of 1080 mJ/cm^2), animals were sacrificed, and the skin lesions were surgically removed at 30 weeks after the last UVB exposure. Tissue sections ($4 \mu\text{m}$) were stained with H&E and examined microscopically as detailed in Materials and Methods. Typical representative pictures are shown.

cysts and pigmented follicular inclusion cysts containing keratinocyte material with increased keratinocyte melanization (Figure 7). Under the UVB exposure protocol used, SKH-1 hairless or littermate nontransgenic mice did not develop tumors or cysts even up to 50 weeks (data not shown).

Because our data demonstrated that limited UVB-exposure (initiating dose) resulted in significant tumorigenesis in ODC-overexpressing mice, it implies that ODC is a key contributor in UVB-mediated responses including the development of skin cancer. Although some earlier

studies have suggested the involvement of ODC during UVB responses such as inflammation and carcinogenesis,^{45–48} the present study used a genetic approach to elucidate the definitive role of this enzyme during photocarcinogenesis and other UV responses.

To further confirm the role of ODC in UVB-mediated skin carcinogenesis, we performed a repeat experiment of limited UVB exposure in which we included an extra group of ODC transgenic mice, which after the last UVB exposure, were given DFMO (1% w/v) in the drinking water. These animals were monitored for up to 30 weeks

Figure 8. Effect of DFMO feeding on the formation of skin lesions in K5/ODC transgenic mice. The mice were divided in three groups of eight mice. The first group of mice did not receive any treatment and served as controls. The second group of animals was subjected to UVB exposure (180 mJ/cm^2 , three times a week for 2 weeks). The third group of animals, after the last UVB exposure, was given DFMO (1% w/v) in the drinking water. The animals were followed for the formation of skin lesions. Details are given in Materials and Methods. Typical representative pictures are shown.

and as shown in Figure 8, none of the ODC transgenic mice was found to develop any kind of epidermal tumor. The formation of pigmented cysts in these mice was also substantially lower with <10 cysts per mouse on test (data not shown). Because DFMO is an irreversible inhibitor of ODC, these data further confirmed that ODC plays a key role in skin carcinogenesis by UV exposure. Recent studies have suggested that ODC is a central convergence point of growth-promoting signals, which via uncontrolled proliferation of initiated cells, may lead to the development of neoplastic conditions.^{44,49} These studies and our present findings suggest that ODC plays a critical role in the promotion phase of skin carcinogenesis by UVB exposure. An important corollary to our finding is that the agents that inhibit ODC could be able to interfere with the development of skin cancers by reversing the promotion phase of the carcinogenesis process. Based on our study, we suggest that to reduce the occurrence of skin cancer, the use of ODC inhibitors in skin care products should be considered.

The exact mechanism by which ODC overexpression results in the development of skin tumors as a result of limited UVB exposure is not known at present. However, on the basis of some recent studies, we believe that the involvement of mitogen-activated protein kinase pathway- and cyclin kinase inhibitor-cyclin-cyclin-dependent kinase machinery in ODC caused increased cellular proliferation of UV-initiated cells during photocarcinogenesis is an intriguing possibility.^{12,18,50,51} A detailed investigation of these pathways is our future goal.

References

- Ananthaswamy HN, Loughlin SM, Cox P, Evans RL, Ullrich SE, Kripke ML: Sunlight and skin cancer: inhibition of p53 mutations in UV-irradiated mouse skin by sunscreens. *Nat Med* 1997, 3:510-514
- Scharffetter-Kochanek K, Brenneisen P, Wenk J, Herrmann G, Ma W, Kuhr L, Meewes C, Wlaschek M: Photoaging of the skin from phenotype to mechanisms. *Exp Gerontol* 2000, 35:307-316
- de Gruijl FR: Skin cancer and solar UV radiation. *Eur J Cancer* 1999, 35:2003-2009
- Brash DE, Ponten J: Skin precancer. *Cancer Surv* 1998, 32:69-113
- Brash DE, Ziegler A, Jonason AS, Simon JA, Kunala S, Leffell DJ: Sunlight and sunburn in human skin cancer: p53, apoptosis, and tumor promotion. *J Invest Dermatol Symp Proc* 1996, 1:136-142
- Mukhtar H, Elmetts CA: Photocarcinogenesis: mechanisms, models and human health implications. *Photochem Photobiol* 1996, 63:355-447
- Parker SL, Davis KJ, Wingo PA, Ries LA, Heath Jr CW: Cancer statistics by race and ethnicity. *CA Cancer J Clin* 1998, 48:31-48
- Sarasin A: The molecular pathways of ultraviolet-induced carcinogenesis. *Mutat Res* 1999, 428:5-10
- Shantz LM, Pegg AE: Translational regulation of ornithine decarboxylase and other enzymes of the polyamine pathway. *Int J Biochem Cell Biol* 1999, 31:107-122
- Pegg AE: Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res* 1988, 48:759-774
- Tabor CW, Tabor H: Polyamines. *Annu Rev Biochem* 1984, 53:749-790
- Gilmour SK, Birchler M, Smith MK, Rayka K, Mostochuk J: Effect of elevated levels of ornithine decarboxylase on cell cycle progression in skin. *Cell Growth Differ* 1999, 10:739-748
- Verma AK: Inhibition of tumor promotion by DL-alpha-difluoromethylornithine, a specific irreversible inhibitor of ornithine decarboxylase. *Basic Life Sci* 1990, 52:195-204
- Slaga TJ, Fischer SM, Weeks CE, Nelson K, Mamrack M, Klein-Szanto AJ: Specificity and mechanism(s) of promoter inhibitors in multistage promotion. *Carcinog Compr Surv* 1982, 7:19-34
- Boutwell RK, Verma AK, Ashendel CL, Astrup E: Mouse skin: a useful model system for studying the mechanism of chemical carcinogenesis. *Carcinog Compr Surv* 1982, 7:1-12
- Yuspa SH, Hennings H, Saffiotti U: Cutaneous chemical carcinogenesis: past, present, and future. *J Invest Dermatol* 1976, 67:199-208
- Choudhary SK, Sharma D, Dixit A: D,L-alpha-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase, induces differentiation in MEL cells. *Cell Biol Int* 1999, 23:489-495
- Flamigni F, Facchini A, Capanni C, Stefanelli C, Tantini B, Caldarella CM: p44/42 mitogen-activated protein kinase is involved in the expression of ornithine decarboxylase in leukaemia L1210 cells. *Biochem J* 1999, 341:363-369
- Meyskens Jr FL, Gerner EW: Development of difluoromethylornithine (DFMO) as a chemoprevention agent. *Clin Cancer Res* 1999, 5:945-951
- O'Brien TG, Megosh LC, Gilliard G, Soler AP: Ornithine decarboxylase overexpression is a sufficient condition for tumor promotion in mouse skin. *Cancer Res* 1997, 57:2630-2637
- Megosh L, Gilmour SK, Rossion D, Soler AP, Blessing M, Sawicki JA, O'Brien TG: Increased frequency of spontaneous skin tumors in transgenic mice which overexpress ornithine decarboxylase. *Cancer Res* 1995, 55:4205-4209
- Kumlin T, Kosma VM, Alhonen L, Janne J, Komulainen H, Lang S, Rytomaa T, Servomaa K, Juutilainen J: Effects of 50 Hz magnetic fields on UV-induced skin tumorigenesis in ODC-transgenic and non-transgenic mice. *Int J Radiat Biol* 1998, 73:113-121
- Heljasvaara R, Veress I, Halmekyto M, Alhonen L, Janne J, Laajala P, Pajunen A: Transgenic mice overexpressing ornithine and S-adenosylmethionine decarboxylases maintain a physiological polyamine homeostasis in their tissues. *Biochem J* 1997, 323:457-462
- Reeben M, Arbatova J, Palgi J, Miettinen R, Halmekyto M, Alhonen L, Janne J, Riekkinen P, Saarma M: Induced expression of neurotrophins in transgenic mice overexpressing ornithine decarboxylase and overproducing putrescine. *J Neurosci Res* 1996, 45:542-548
- Hansen LA, Tennant RW: Follicular origin of epidermal papillomas in v-Ha-ras transgenic TG.AC mouse skin. *Proc Natl Acad Sci USA* 1994, 91:7822-7826
- Spalding JW, Momma J, Elwell MR, Tennant RW: Chemically induced skin carcinogenesis in a transgenic mouse line (TG.AC) carrying a v-Ha-ras gene. *Carcinogenesis* 1993, 14:1335-1341
- Smith MK, Trempus CS, Gilmour SK: Co-operation between follicular ornithine decarboxylase and v-Ha-ras induces spontaneous papillomas and malignant conversion in transgenic skin. *Carcinogenesis* 1998, 19:1409-1415
- Das M, Mukhtar H, Bik DP, Bickers DR: Inhibition of epidermal xenobiotic metabolism in SENCAR mice by naturally occurring plant phenols. *Cancer Res* 1987, 47:760-766
- Agarwal R, Katiyar SK, Zaidi SI, Mukhtar H: Inhibition of skin tumor promoter-caused induction of epidermal ornithine decarboxylase in SENCAR mice by polyphenolic fraction isolated from green tea and its individual epicatechin derivatives. *Cancer Res* 1992, 52:3582-3588
- Wu L, Levine AJ: Differential regulation of the p21/WAF-1 and mdm2 genes after high-dose UV irradiation: p53-dependent and p53-independent regulation of the mdm2 gene. *Mol Med* 1997, 3:441-451
- Kulms D, Schwarz T: Molecular mechanisms of UV-induced apoptosis. *Photodermatol Photoimmunol Photomed* 2000, 16:195-201
- Soler AP, Gwendolyn G, Megosh LC, O'Brien TG: Modulation of murine hair follicle function by alterations in ornithine decarboxylase activity. *J Invest Dermatol* 1996, 106:1108-1113
- Iwata S, Sato Y, Asada M, Takagi M, Tsujimoto A, Inaba T, Yamada T, Sakamoto S, Yata J, Shimogori T, Igarashi K, Mizutani S: Anti-tumor activity of antizyme which targets the ornithine decarboxylase (ODC) required for cell growth and transformation. *Oncogene* 1999, 18:165-172
- Gilmour SK, O'Brien TG: Regulation of ornithine decarboxylase gene expression in normal and transformed hamster embryo fibroblasts following stimulation by 12-O-tetradecanoylphorbol-13-acetate. *Carcinogenesis* 1989, 10:157-162
- Sistonen L, Holttä E, Lehtola L, Alitalo K: Activation of the neu tyrosine kinase induces the fos/jun transcription factor com-

- plex, the glucose transporter and ornithine decarboxylase. *J Cell Biol* 1989, 109:1911–1919
36. Auvinen M, Paasinen A, Andersson LC, Holtta E: Ornithine decarboxylase activity is critical for cell transformation. *Nature* 1992, 360:355–358
37. Holtta E, Auvinen M, Andersson LC: Polyamines are essential for cell transformation by pp60v-src: delineation of molecular events relevant for the transformed phenotype. *J Cell Biol* 1993, 122:903–914
38. Astrup EG, Boutwell RK: Ornithine decarboxylase activity in chemically induced mouse skin papillomas. *Carcinogenesis* 1982, 3:303–308
39. Boutwell RK, O'Brien TG, Verma AK, Weekes RG, DeYoung LM, Ashendel CL, Astrup EG: The induction of ornithine decarboxylase activity and its control in mouse skin epidermis. *Adv Enzyme Regul* 1978, 17:89–112
40. O'Brien TG: The induction of ornithine decarboxylase as an early, possibly obligatory, event in mouse skin carcinogenesis. *Cancer Res* 1976, 36:2644–2653
41. DiGiovanni J: Multistage carcinogenesis in mouse skin. *Pharmacol Ther* 1992, 54:63–128
42. Yuspa SH: The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis—thirty-third G. H. A. Clowes Memorial Award Lecture. *Cancer Res* 1994, 54:1178–1189
43. Puignero V, Turull A, Queralt J: Arachidonic acid (AA) and tetradecanoylphorbol acetate (TPA) exert systemic effects when applied topically in the mouse. *Inflammation* 1998, 22:307–314
44. Auvinen M: Cell transformation, invasion, and angiogenesis: a regulatory role for ornithine decarboxylase and polyamines? *J Natl Cancer Inst* 1997, 89:533–537
45. Kumlin T, Alhonen L, Janne J, Lang S, Kosma VM, Juutilainen J: Epidermal ornithine decarboxylase and polyamines in mice exposed to 50 Hz magnetic fields and UV radiation. *Bioelectromagnetics* 1998, 19:388–391
46. Katiyar SK, Korman NJ, Mukhtar H, Agarwal R: Protective effects of silymarin against photocarcinogenesis in a mouse skin model. *J Natl Cancer Inst* 1997, 89:556–566
47. Rosen CF, Gajic D, Drucker DJ: Ultraviolet radiation induction of ornithine decarboxylase in rat keratinocytes. *Cancer Res* 1990, 50:2631–2635
48. Lowe NJ, Breeding J: Antiinflammatory drug effects on ultraviolet light-induced epidermal ornithine decarboxylase and DNA synthesis. *J Invest Dermatol* 1980, 74:418–420
49. Marton LJ, Pegg AE: Polyamines as targets for therapeutic intervention. *Annu Rev Pharmacol Toxicol* 1995, 35:55–91
50. Marty C, Mori G, Sabini L, Rivarola V: Effects of alpha-difluoromethylornithine on the cyclin A expression in Hep-2 cells. *Biocell* 2000, 24:49–52
51. Parekh VV, Hoffman JL, Younoszai MK: Role of tyrosine kinase, ODC, and p34cdc2 kinase and cyclin B-associated cdc2 in jejunal enterocyte proliferation, maturation, and exfoliation in diabetic and DFMO-treated rats. *J Invest Med* 1999, 47:397–404