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ENHANCED PAPILLOMA FORMATION IN RESPONSE TO SKIN TUMOR PROMOTION IN TRANSGENIC MICE OVEREXPRESSING THE HUMAN ORNITHINE DECARBOXYLASE GENE

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SUMMARY: We have studied the induction of papilloma formation in response to skin tumor promotion in transgenic mice overexpressing the human ornithine decarboxylase gene and in their nontransgenic littermates. The transgenic animals displayed a basal epidermal ornithine decarboxylase activity that was nearly 20 times higher than in their nontransgenic littermates. A single topical application of 12-*O*-tetradecanoylphorbol-13-acetate induced a much more profound and longer-lasting increase in transgene-derived ornithine decarboxylase activity in comparison with the endogenous enzyme activity. Initiation of skin tumorigenesis with a single topical application of dimethylbenz[a]antracene followed by twice-weekly application of 12-*O*-tetradecanoylphorbol-13-acetate resulted in the appearance of first papillomas both in nontransgenic and transgenic animals by week 7. However, after 11 weeks of 12-*O*-tetradecanoylphorbol-13-acetate application, the number of papillomas per animal was almost 100 % higher in the transgenic animals than in their nontransgenic littermates. These results indicate that an overexpression of epidermal ornithine decarboxylase confers a growth advantage on skin tumors *in vivo*.

Although ornithine decarboxylase (EC 4.1.1.17) is considered to be a growth-related enzyme displaying features resembling those of the protooncogenes (for ref. see 1), relatively little experimental evidence directly supports this view. Most of the studies exploiting the role of high ornithine decarboxylase in growth potential have been carried out with cultured cells overexpressing the enzyme. We found that overexpression of ornithine decarboxylase owing to gene amplification conferred growth advantage on mouse leukemia and myeloma cells as manifested by the ability to grow in semisolid media (2). Similarly, others (3) reported that overproduction of transfected human ornithine decarboxylase gene in Chinese hamster ovary cells resulted in faster growth of the stable transfectants in comparison with the wild-type cells. According to retrovirus-mediated transfection experiments recently reported (4), an overexpression of murine ornithine decarboxylase in mouse or rat fibroblasts did not alter the morphology or growth properties of the infected cells but rendered the rat fibroblasts displaying high ornithine decarboxylase activity markedly more

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susceptible to transformation by an activated c-H-*ras* oncogene. This finding may indicate that ornithine decarboxylase can cooperate with activated oncogenes (4).

Even though the evidence that a high ornithine decarboxylase activity provides growth advantage to or induces transformation of mammalian cells is still circumvential, much stronger evidence supports the view that ornithine decarboxylase and hence the formation of putrescine and spermidine are *required* for mammalian cell proliferation to occur. Inhibitors of ornithine decarboxylase, such as α-difluoromethylornithine, have been used in the treatment of experimental and even clinical cancer and, more importantly, have been employed as experimental chemopreventive agents (for ref. see 1). Probably best documented examples of the latter use is the prevention of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) -promoted skin tumor formation in mice by α-difluoromethylornithine (5-7). The drug similarly inhibited skin tumor promotion by chrysarobin (8) and skin carcinogenesis induced by ultraviolet irradiation (9).

We have recently produced a number of transgenic mouse lines harboring the human ornithine decarboxylase gene in their genome. These animals overexpress the transgene virtually in all of their tissues (10, 11) in a position-independent, gene copy number-dependent fashion (12). As these transgenic animals also display a very high epidermal ornithine decarboxylase activity, we have now used them to elucidate the possible role of high ornithine decarboxylase in skin tumor promotion. Although somewhat fewer transgenic animals (80 % vs 100 %) developed papillomas in response to the tumor promotion, the average number of papillomas per animal was almost twice as high in the transgenic animals in comparison with their nontransgenic littermates. This is probably the first piece of experimental evidence indicating that a high tissue ornithine decarboxylase activity enhances chemical tumor promotion.

MATERIALS AND METHODS

The transgenic animals used in this study were female belonging to the K2 line harboring 24 copies of the human ornithine decarboxylase gene (12). These animals overexpress the transgene in most of their tissues (11). Nontransgenic female littermates served as controls.

The skin tumorigenesis was initiated by a topical application of 200 nmol of dimethylbenz[a]antracene in acetone onto shaved dorsal skin under subdued lightning. Two weeks after the initiation, twice-weekly applications of TPA (10 nmol in acetone) were started. This protocols closely follows that presented in [13]. In case of hair regrowth, the shaving was repeated. The incidence of papillomas was recorded weekly. Histological examination of the papillomas was performed at week 13. Both syngenic and transgenic animals were divided into 4 groups (8 to 10 animals in each) as follows: untreated controls, dimethylbenz[a]antracene treatment followed by twice-weekly applications of acetone, initiation with acetone followed by twice-weekly promotion with TPA and initiation with demethylbenz[a]antracene followed by twice-weekly promotion with TPA.

The activity of ornithine decarboxylase was assayed essentially as in [14].

For statistical analyses, tow-tailed Student's t-test or analysis of variance were used.

RESULTS

The transgenic animals displayed a basal epidermal ornithine decarboxylase activity that was nearly 20 times higher than that in their nontransgenic littermates (214 ± 71 versus 12 ± 5 pmol/mg protein per h; $p < 0.05$). Fig. 1. depicts the induction of epidermal ornithine decarboxylase activity in response to a single topical TPA application in nontransgenic and transgenic animals. While the enzyme activity was peaking at the same time (6 h after the application) in both animal groups one may notice that the maximum response in the transgenic animals was more profound and also longer-lasting (Fig. 1). The transgene-derived activity was still significantly higher than the endogenous activity at 24 h after the application.

While no tumors developed in animals (syngenic or transgenic) treated with the initiating compound only or with TPA without the initiation, the first tumors appeared by week 7 in animals initiated with dimethylbenz[a]antracene and promoted with TPA. Ultimately 10/10 (100 %) of the nontransgenic and 7/9 (80 %) of the transgenic animals developed papillomas. The two pigmented transgenic animals with no papillomas, however, developed early (by week 13) cutaneous melanosis characterized by distinct pigmented spots over the dorsal skin. Histological examination of these spots revealed mainly macrophages loaded with melanin pigment. There were alltogether 4 pigmented mice in both groups (the rest were albinos). By the end of the examination period (week 16) 3/4 of the pigmented transgenic and 2/4 of the pigmented nontransgenic mice developed cutaneous melanosis.

Fig. 2 depicts the cumulative incidence of papillomas in nontransgenic and transgenic animals. As shown in the picture, after week 11 the transgenic animals

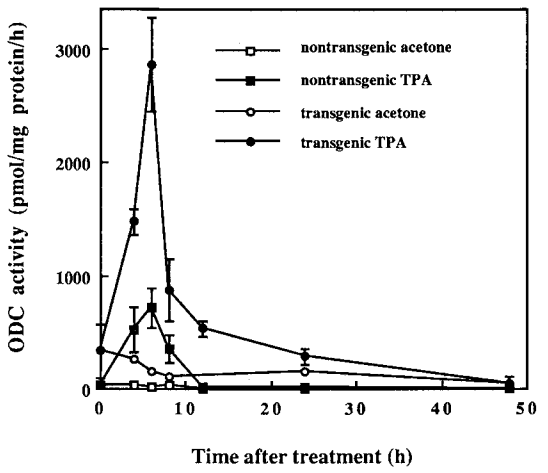

Fig. 1. Effect of a single application of TPA on epidermal ornithine decarboxylase (ODC) activity in nontransgenic and transgenic mice. The results are means ± S.E.M. obtained from 3 animals in each group.

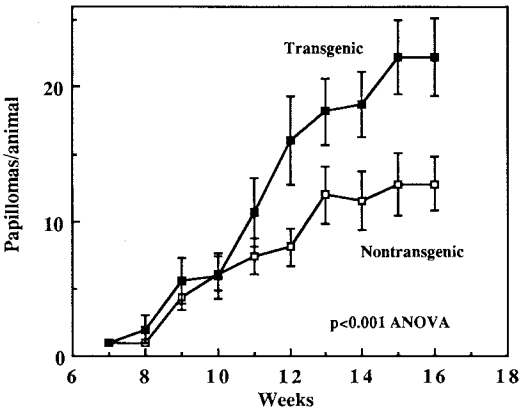

Fig. 2. Formation of skin papillomas in response to tumor promotion in nontransgenic and transgenic mice. The tumorigenesis was initiated with a single topical application of dimethylbenz[a]antracene followed by twice-weekly TPA applications. Seven to nine animals in each group. The vertical bars represent standard errors of the mean. ANOVA, analysis of variance.

had nearly twice as many papillomas than their nontransgenic littermates. The difference between the groups was statistically highly significant ($p < 0.001$; analysis of variance).

Histological examination performed at week 13 revealed that the tumors of both groups represented benign papillomas with practically no dysplasia. This was not unexpected as the protocol used for the tumorigenesis favors the formation of benign tumors (8). In any event, these results strongly support the view that high ornithine decarboxylase activity enhances skin tumorigenesis.

DISCUSSION

Although our earlier studies have suggested that high ornithine decarboxylase activity, such as might result from gene amplification, may give growth advantage to tumor cells as manifested as enhanced aggressiveness (15) or an ability to form colonies in semisolid media (2), the present results indicate for the firs time that high tissue ornithine decarboxylase activity also enhances tumor promotion *in vivo*. The exact pathophysiological mechanisms involved in the multistage skin tumorigenesis are not known, but it has been suggested that point mutations of the H-*ras* oncogene are associated with the tumor initiating event produced by dimethylbenz[a]antracene (8). Interestingly, an overexpression of ornithine decarboxylase has been shown to enhance fibroblast transformation by activated H-*ras* oncogene (4). It is thus possible that ornithine decarboxylase cooperate with some other agents, such as oncogene products, in the process of skin carcinogenesis. The fact, however, remains that in spite of the high epidermal ornithine decarboxylase activity both the initiation and promotion are needed for the

tumorigenesis to occur. This was demonstrated by the lack of tumor development in the transgenic animals in response to the initiation or promotion alone.

Our present results somewhat disagree with those recently reported by Imamoto et al. (16). The latter authors compared different mouse strains as regards the magnitude of TPA-induced ornithine decarboxylase activity and the responsiveness to tumor promotion, but did not find any correlation between these variables (16). An explanation for this discrepancy may just be the fact the differences between ornithine decarboxylase activities among the natural mouse strains were small in comparison with our transgenic mice.

These results also substantiate the view that high ornithine decarboxylase activity may be a risk factor for the development of cancer under pathological conditions, such as familial colonic polyposis (17, 18).

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