A-4.5.2 The Combined Effect of Ultraviolet Irradiation and Toxic Chemicals on the Skin Tumorigenesis: Role of Oxidative Stress

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ABSTRACT

Based upon the earlier findings that UV as well as quite a few carcinogens exert their harmful effects through oxidative stress, we focus upon the oxidative stress and skin cancers in our study. We have used metallothionein knock out [MT (-/-)] mice that are susceptible to oxidative damage, and had them exposed to UVB irradiation and 7,12-dimethylbenz[a]anthracene (DMBA), a well-known carcinogen. The acute exposure to UVB showed that UVB (5 KJ/m^2) induced same skin thickness response in both strains of mice. DMBA induced strong acute inflammatory responses in the skin of MT (-/-) mice than wild-type [MT (+/+)] mice. The skin damage was more serious after short time of combined exposure. Moreover, Topical application of administration of DMBA resulted in an increase in papilloma in MT (-/-) mice skin in a dose-dependent manner after 14 weeks of treatment. However, no change was observed in the skin of MT (+/+) mice by DMBA treatment. The long-term UVB irradiation enhanced the skin tumor incidence caused by DMBA. These results suggested MT plays a defensive role against skin tumorigenesis of DMBA and UVB. The experimental result obtained so far provides that genetic traits on sensitivity to oxidative stress should be thought for the health risk assessment of human populations.

Keywords Ultraviolet irradiation, 7,12-Dimethylbenz[a]anthracene, Skin carcinogenesis, Oxidative stress, Metallothionein.

1. Introduction

The incidence of human cancer has increased dramatically over the last several decades[1]. The environmental causes of cancer, such as polycyclic aromatic hydrocarbons (PAH) and excessive exposure to sunlight UV, are responsible for the vast majority of skin cancers[2]. Epidemiological data indicated that the risk of non-melanoma cancers is correlated with cumulative lifetime UV exposure. It is assumed that each 1 % decrease in stratospheric ozone leads to about a 2 % increase in the biological censequences[3]. In the processes of carcinogenesis, the oxidative stress and free radical pathway have been indicated in the primary genotoxicity and endogenous antioxidant system are important in defense against carcinogenesis[4].

Metallothionein (MT) is a cysteine–rich, low molecular weight, metal-bindingProtein[5]. Recently, the role of MT acting as free radical scavenger and antioxidant ability are very attractive[6]. We selected the transgenic mice that MT gene had been knock out [MT (-/-)] and shown that these mice are sensitive to 7,12-dimethylbenz[a]anthracene (DMBA), a carcinogenic PAH, induced skin tumorigenesis[7]. In this year, the relationship of inflammatory damage and tumorigenesis have been studied In present report, the effects of UV or DMBA alone, and combination exposure of them were collected and presented.

2. Materials and methods

Chemicals and the UVB source

DMBA was purchased from Sigma Chemical Co. (St. Louse, MO, U.S.A.). The UVB source consisted of bank of four UV lamps (FLISE, 280-320 nm, peak wave length at 312 nm, NIS Company, Japan). The irradiated lever at 312 nm was monitored daily by a spectroradiometer (RMX, Model ATR-3w, Atto Co., Japan), and the sensor of which was placed at the same level of mice.

Transgenic mice

MT (-/-) mice and MT (+/+) (C57BL6/OLA129) mice were kindly provided by Dr. A Choo[8]. Ten week-old female mice were used and the dorsal skin of each mouse was shaved using hair clippers under pentobarbital anesthesia.

Short-term UVB irradiation

The UVB exposure started one week after hair shaving. The UVB irradiation dose was selected to be 2.5, 5.0 and 10.0 KJ/m^2, respectively. Before UVB irradiation, the back skin fold thickness measured by a caliper (Pocket Thickness Gage, Mitutoyo, Japan). Mice were received three exposures weekly for 2 weeks. On selected days (24 hr after first, third and sixth UVB irradiation), the mice were sacrificed by an overdose of ether. After the skin thickness measurement, this portion of skin was collected for the histological examination.

Short –term combined exposure

The mice were topically administrated on their shaved dorsal skin 100, 250 and 500 μg of DMBA in 100 μl acetone per mouse. At one week after DMBA topical application, UVB (5 KJ/m^2) irradiation started. Mice were received three exposures weekly at every other weekday. The mice sample selected 24 hr after first UVB and third UVB irradiation.

Long–term combined exposure

The mice were topically administered on their shaved dorsal skin 100, 250 and 500

μg of DMBA per mouse and the UVB (5.0 KJ/m^2) exposure started one week after the DMBA treatment. Mice were received three exposures weekly for 13 weeks. At week 14 after DMBA treatment and 24 hr after last UVB irradiation, the skin tumors were counted.

Histological and biochemical examination

Skin specimens were fixed in neutral-buffered formalin solution and processed for paraffin embedding. Skin sections (5 μm) were prepared and placed on a glass slide for hematoxylin and eosin.

3. Results

Short-term UVB irradiation

After the UVB irradiation, the skin thickness was increased in the UVB dose-dependent manner in both MT (-/-) mice and MT (+/+) mice (Fig. 1). The skin fold thickness reached maximum after third UVB irradiation. There were no significant differences between MT (-/-) mice and MT (+/+) mice at 2.5 or 5.0 KJ/m^2 of UVB, but at 10.0 KJ/m^2 of UVB the skin was more thick in MT (+/+) mice than MT (-/-) mice. As at 5.0 KJ/m^2 of UVB irradiation, there was no significant differences between both kinds of mice, we selected this dose of UVB for combined experiment.

Short-term combined exposure

We selected the skin ulceration as the acute irritant inflammatory response (Table 1). DMBA acted as a strong irritant agent in MT (-/-) mice whereas the skin ulcerlation was not observed in MT (+/+) mice. The ulceration first appeared 5 days after treatment and reached maximum by 10 to 14 days. This delay in the onset of ulceration may suggest that DMBA produced contact hypersensitivity. Some acute ulceration persisted and became chronic ulceration. The acute skin ulceration caused by DMBA correlated to the tumor formation.

Moreover, we observed histopathological changes in the skin of mice treated with combined exposure of 500 μg of DMBA and 5 KJ/m^2 of UVB (Fig. 2). At 8 days after DMBA (500 μg) treatment, the proliferation and enlargement of nuclei of epidermis cell were observed in both kinds of mice. At 12 days after DMBA treatment, the disorganization of epidermis (erosion) was observed in all of the MT (-/-) mice, while the recovery from enlargement of nuclei cells to control level was observed in all of the MT (+/+) mice. The connective tissue of ulcer skin was more dense in combined exposure than DMBA treatment alone in MT (-/-) mice (Fig. 2A to D). In MT (+/+) mice, the skin thickness was induced after combined exposure and the level was higher than those of DMBA alone (Fig. 2G,H).

Long-term combined exposure

The results of skin tumor induction by either DMBA alone or DMBA plus UVB irradiation are shown in Table 2 and Fig. 3. There was a statistically significant dose-dependent elevation of tumorigenesis by DMBA in MT (-/-) mice, whereas no tumor was observed in MT (+/+) mice by DMBA treatment. Pretreatment of skin by DMBA followed by UVB irradiation enhanced the incidence of skin tumor by 10 to 20 %. At a dose of 500 μg DMBA, UVB irradiation enhanced the incidence of skin tumor to 54 % in MT (-/-) mice. At MT (+/+) mice, the enhancement of tumor formation was to 20 %. All the tumors were classified histologically as papilloma. No skin tumor was found in MT (-/-) and MT (+/+) mice treated with UVB alone.

The skin ulceration was selected as a biomarker of response to DMBA and/or UVB (Table 2). The mice (-/-) mice were sensitive to DMBA treatment or DMBA plus UVB irradiation compared with those of MT (+/+) mice. The UVB irradiation enhanced the formation of skin ulcer initiated by DMBA in both MT (-/-) and MT (+/+) mice. The inflammatory response by UVB treatment only was modest and the epidermal thickness in which cell proliferation was the major type.

4. Discussion

After UVB irradiation, the skin thickness and proliferation of epidermis were observed. DMBA is a skin irritant and UVB can promote DMBA effect on the skin. The present study demonstrated that MT (-/-) mice were more sensitive to the skin damage as well as skin tumorigenesis caused by DMBA alone or by combined exposure of DMBA and UVB than the MT (+/+) mice. The UVB irradiation enhanced the dibenzo[a]pyrene skin tumorigenesis in the mice and DMBA-indued melanocyte tumors in hairless mice[9,10]. The UVB irradiation also enhanced the DMBA-induced lesions in human skin grafted to mice[11]. The combined effect may be mediated by several possible mechanisms including promoting direct action of reacted DMBA on cell[12], generating reactive free radials and reactive oxygen species (oxidative stress) as well as generating prostaglandins, histamine, leucotrienes, and other inflammatory mediators[13].

Oxidative stress is refereed to as an imbalanced condition of prooxidant/antioxidant equilibrium. There is the antioxidant system in the skin and if the antioxidant system fails and reactive oxidants exceeds the antioxidant system, the oxidative stress will come and oxidative injury will result. MT is antioxidant in the cell, the absence of MT will make mouse loss the balance of antioxidant statue and weaken the ability of anti-against oxidative stress. The higher lipid peroxide levels in the control MT (-/-) mice suggested that the MT null condition was in a kind of oxidative stress condition compared with MT (+/+) mice before exposure to chemicals and UV irradiation (our unpublished data).

In conclusion, DMBA produced a dose-dependent manner of skin tumor response in MT (-/-) mice while no tumor occurred in MT (+/+) mice. UVB irradiation promoted the tumor formation induced by DMBA. Inflammatory response, which is mainly mediated by active oxygen radicals, plays an important role in DMBA and UVB combinative effect on skin. To evaluate health risk assessment of UVB irradiation, it is necessary to consider other coexisting environmental chemicals as well as the predisposition of human populations.

5. References

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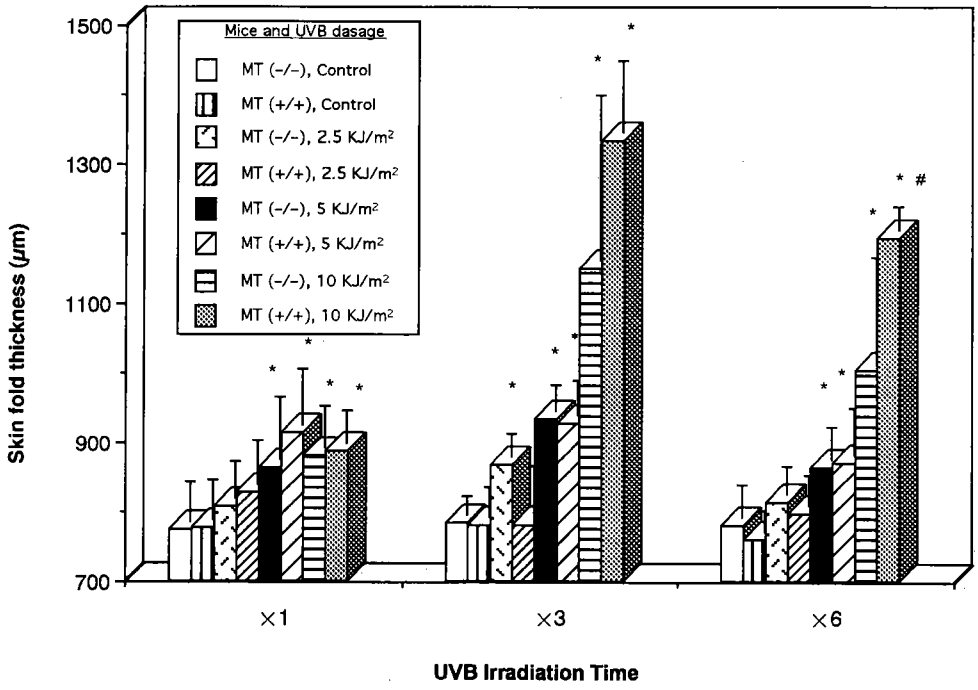

Fig. 1. Skin Inflammatory Response after Short-term UVB Irradiation in MT (–/–) mice and MT (+/+) Mice.
*** Significantly different from the corresponding control mice (P<0.05, n=5, mean ± SD).**
 # Significantly different from the corresponding MT (-/-) mice (P<0.05, n=5, mean ± SD).

Table 1 Mouse Skin Acute Inflammatory Response Caused by a Single Application of Different Doses of DMBA or DMBA Followed Three Times of UVB Irradiation

Treatment	Ulceration (%)*	
	MT(-/-)	MT(+/+)
Control	0	0
DMBA(100 μg)	0	0
DMBA(250 μg)	50	0
DMBA(500 μg)	91	0
UVB	0	0
DMBA(100 μg) + UVB	0	0
DMBA(250 μg) + UVB	50	17
DMBA(500 μg) + UVB	100	30

* The skin ulceration was observed at 24 hr after the third UVB irradiation.

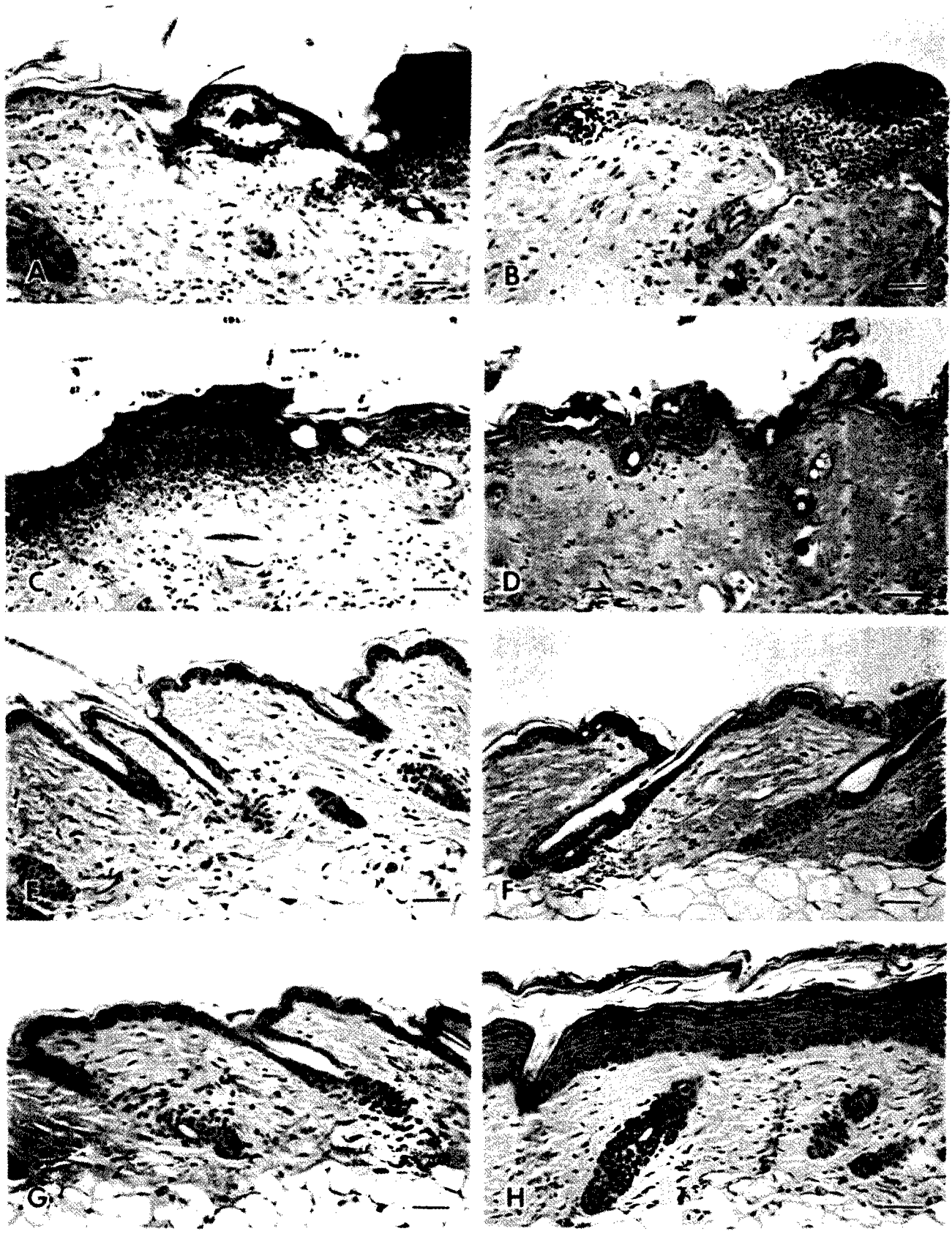

Fig. 2. Histopathological Changes in the Skin of Mice Treated with Combined Exposure of 500 μg of DMBA and 5 KJ/m² of UVB with Hematoxylin and Eosin Staining. A, B, C and D show MT (–/–) mice. E, F, G and H exhibit MT (+/+) mice. A, C, E and G, DMBA alone treatment. B, D, F and H, combined exposure. A, B, E and F show 24 hr after 1st UVB irradiation. C, D, G and H show 24 hr after 3rd UVB irradiation. Bar, 100 μm.

Table 2 Mouse Skin Tumor and Skin Chronic Inflammatory Response caused by a Single Application of Different Doses of DMBA or DMBA Followed by Repeated UVB Irradiation for 13 Weeks

Treatment	Tumor incidence (%)		Ulceration (%)	
	MT(-/-)	MT(+/+)	MT(-/-)	MT(+/+)
Control	0	0	0	0
DMBA(100 μg)	0	0	0	0
DMBA(250 μg)	20	0	20	0
DMBA(500 μg)	33[a,b]	0	24[a,b]	0
UVB	0	0	0	0
DMBA(100 μg) + UVB	0	0	0	0
DMBA(250 μg) + UVB	30[a]	0	20	17
DMBA(500 μg) + UVB	54[a]	20[c]	64[a,b,c]	10

a-c: Different analysis by chi-square test (n=11 to 20).

a: $P<0.05$ compared with control or UVB treated mice.

b: $P<0.05$ compared with corresponding MT (+/+) mice.

c: $P<0.05$ compared with corresponding DMBA treated mice.

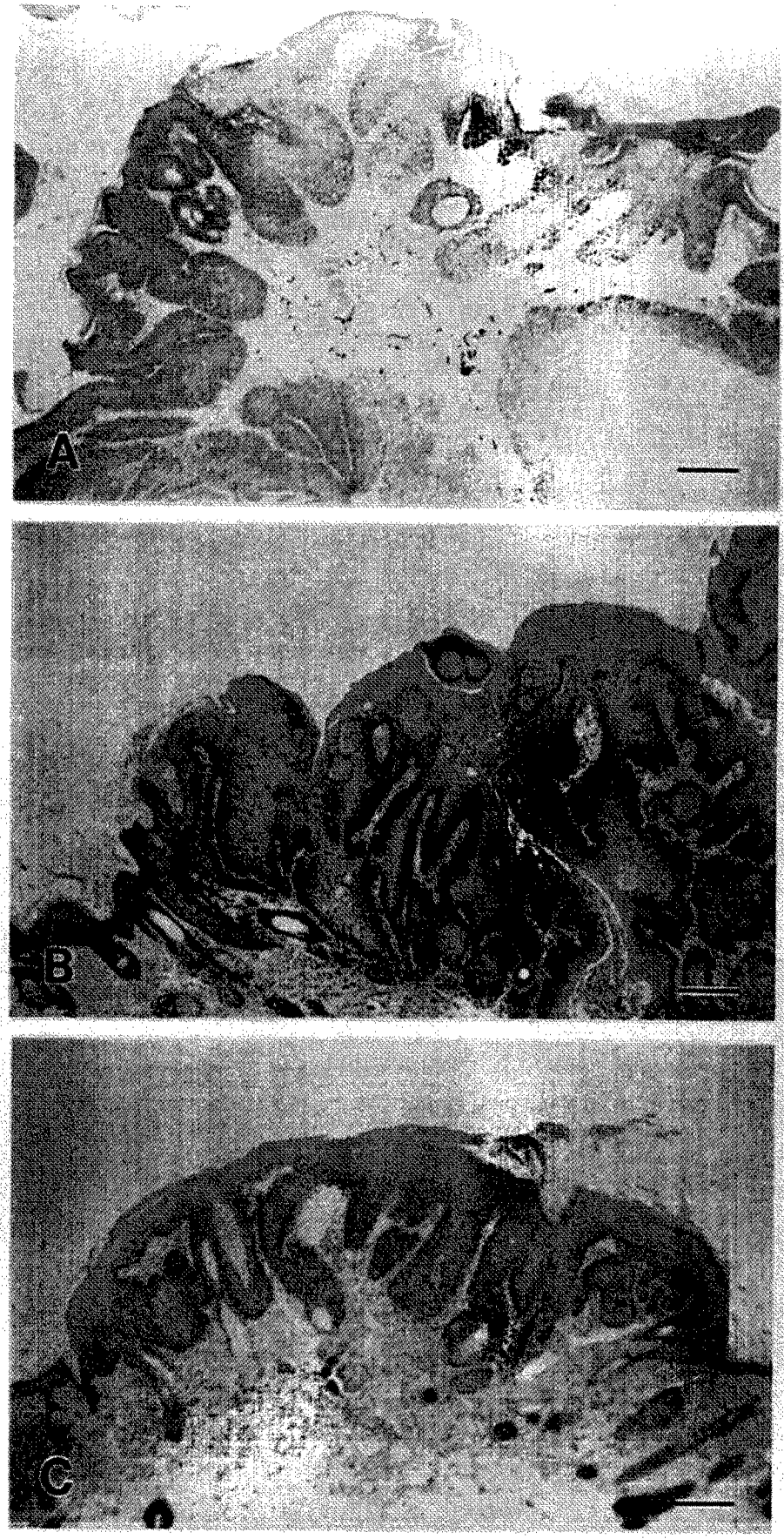

Fig. 3. Skin Tumor in MT (-/-) and MT (+/+) Mice after Long-term Combined Exposure.
A: MT (-/-) mice treated with DMBA. *B*: MT (-/-) mice treated with DMBA plus UVB.
C: MT (+/+) mice treated with DMBA plus UVB. *Bar*, 200 μm.