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7, 12-dimethylbenz [a] anthracene (DMBA)/Ultraviolet radiation (UVB)-induced skin cancer on mouse model

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Introduction

The skin is the largest organ of the mammalian body, made up of multiple layers, which include the epidermis, dermis, and subcutis.[1] These have major functions in several biological processes like environmental barrier, tissue regeneration, hair cycling, and wound repair. In recently, the environmental issues are harmful trouble effects of human activities and human healthy. Worldwide cancer Incidence statistics have been reported increasing every year including skin cancer. They are commonly reported in Caucasian and Asian.[4] Typically, skin cancer is uncommon in Thailand but the southern have been reported the highest incidence rate. They cause of the death of skin cancer's patients due to these can invades to other parts of the body which lead to the secondary cancer. The importance factor is UVB, chronic UVB exposure can disturb the balance of homoeostatic regeneration of epidermis and lead to enhanced proliferation of keratinocytes as known as epidermal hyperplasia. This process responses to repeat UVB exposure for prevent a deeply skin cell. Moreover, irradiation of chronic UVB cause accumulated DNA damage and pro-inflammatory that leads to permanent DNA mutation. Normally, DNA repair processes can eliminate DNA mutation when mechanism defense and lose balance of elimination which causes the development of skin cancer.

The mouse model of multi-stage chemical carcinogenesis represents one of the best established *in vivo* models for study of the stepwise development of skin cancer.[2, 6, 7] In this study, mouse model was designed mimic with human activities. Tumor Initiation stage, mice are applied with carcinogens that ultimately leads to DNA mutation in normal skin cells. Tumor promotion stage, UVB irradiation are almost represented to tumor promoting agent. Chronic UVB irradiation causes initiated cells giving rise to premalignant that as a sign of transduction to tumor progression stage, the formation of tumor is completely developed to malignant that could penetrate deeply into dermis layer.[3] We describe the experimental approaches used in our laboratory to induction of skin cancer. 7, 12-dimethylbenz [a] anthracene (DMBA) were representing carcinogen that initiated normal cells in initiation stage.[6, 9] Mice were irradiated chronic UVB at the appropriate dose and times for promotion initiated cells to cancerous cells.[4, 5, 8] Skin mouse tissues were analyzed microscopic structure of skin using histological techniques.

Methods

Design experiment in mouse model

Animal care and handling were approved by Naresuan University Animal Ethics Committee (Approval Code 580805). Male ICR mice (3 weeks old) were purchased from National Laboratory Animal Center (Mahidol University, Nakhon Pathom, Thailand). Mice were housed with free access to food and water in 1 week under controlled conditions of temperature (25°C ±2°C), humidity (50±10 %) and light (12 light:12 dark). Three days before starting of experiment, mice were shaved dorsal hair in 3 X 3 cm^2 by using 8% sodium sulphide x-hydrate solution.[5, 8] Mice of all the groups were monitored and weighed daily before administration of samples.

Animal Treatment

Male ICR mice were divided into 2 groups. Group I (normal; n = 5), mice were non-DMBA/UVB-induced skin cancer and housed with free access to food throughout the experimental period. Group II, mice were initiated skin cancer using application 7, 12-dimethylbenz [a] anthracene (DMBA) (Sigma Chemicals Co, St Louis, USA) at concentration of 200 nmol in 0.2 ml of acetone. [6, 9] One week after initiation, Mice in group II

were irradiated with UVB that was supplied by an array of Toshiba FL8BLB lamps (Toshiba, Japan) for 16 weeks (3 times a week). The intensity of irradiation was progressively increased, from 54 mJ/cm^2 per exposure at week 1 to 126 mJ/cm^2 at week 3. [5, 8]

Skin tissues frozen sections and Histology

After UVB exposure for 16 weeks, mice were sacrificed with overdose of sodium pentabarbital (100 mg/kg, IP). Dorsal skin were quickly removed and fixed with frozen medium (Leica, Milton Keynes, UK) following as frozen section fixation protocol. 8 µm frozen skin sections were cut by using cryotome (Leica, Milton Keynes, UK).[5] Section tissues were stained using Hematoxylin and eosin staining and were visualized under polarized light microscope and camera (Axio Observer Z1, Carl Zeiss Microscopy Ltd., Cambridge, UK).

Results

Skin morphology

After 16 weeks, the appearance of skin morphology were visualized and compared to non-DMBA/UVB-induced skin cancer group or normal group (figure 1 (A)). Normal group appeared skin smoothness. in the othert hand, mice were DMBA/UVB-induced skin cancer that received only deionized water showed winkles, roughness, wounding and node (figure 1 (B)). In addition, chronic exposure to UVB can defense capacities, resulting in premature skin aging and skin damage.[5]

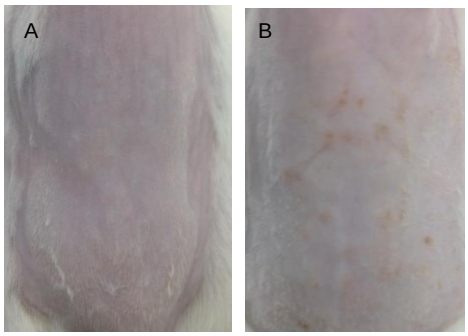

Figure 1. Skin morphology of normal group, (A) and DMBA/UVB-induced skin cancer on mice that orally administrated deionized water, (B).

Effect of DMBA/UVB-induced skin cancer on mice

DMBA/UVB-induced skin cancer on mice was orally administrated only deionized water during promoted UVB for 16 weeks. Light micrograph of tissue stained with hematoxylin and eosin to show the epidermal skin characteristic of normal group in figure 2 (A). In mice induced skin cancer with DMBA and repeated UVB irradiation, the dermal morphology seemed to be proliferate abnormal cell around root hair follicles compared to normal group. In this study, mice were applied DMBA in subcarcinogenic dose only one time for initiation skin cells. Figure 2 (B), repeated UVB irradiation caused over-proliferation of keratinocytes in epidermis, so-called epidermal hyperplasia. In dermis layer, over-proliferation of keratinocytes were increased around root hair follicles and sebaceous glands, so-called acanthosis and atypical nuclei that a sign as skin cancer. Thus, promotion by UVB irradiation can stimulate the initiated skin cells to cancerous cells.

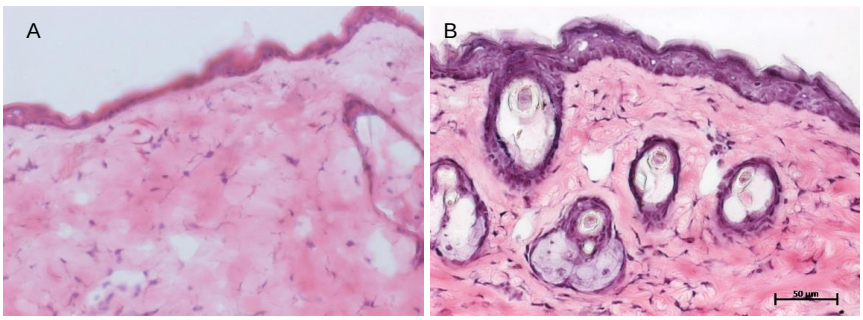

Figure 2. Light micrograph (at magnification, 40x) of tissue stained with hematoxylin and eosin to show the epidermal skin characteristic of normal group, (A) and DMBA/UVB-induced skin cancer on mice that orally administrated deionized water, (B).

Discussion

In this study, Mouse skin model were developed with the induction of single subcarcinogenic dose of DMBA and chronic UVB irradiation for 16 weeks. The appearances of skin morphology were visualized in both groups. Normal group had skin smoothness while DMBA/UVB-induced skin cancer on mice group had winkles and roughness. *Leerach, et al.* found that UVB caused skin roughness, redness and epidermal hyperplasia. However, they irradiated only UVB on control group at appropriate dose for 12 weeks. Their studies support our results in the effect of UVB irradiation in skin morphology. Moreover, skin morphology on DMBA/UVB-induced skin cancer group were appeared wounding, node and tumors that as a sign of formation of skin cancer. Chronic UVB irradiation can defense capacities and nucleotides in DNA stand likely absorb UVB protons, resulting in accumulation DNA damage in skin cells. For clearly study, we evaluated skin tissue using Hematoxylin and eosin staining and visualized under polarized light microscope. Light micrograph of tissue stained showed the epidermal hyperplasia and atypical nuclei around root hair follicles. These apparitions were related with properties of DMBA that can penetrate and accumulate in root hair follicles. DMBA was catalyzed by microsomal enzymes in skin cells and became to secondary metabolites that adducts in DNA stand. DNA adduction causes permanent DNA mutation, over-proliferation of keratinocytes and the development of skin cancer.[6, 9] When DNA mutations occurs in specific DNA sequence, they can loss controllable cell proliferation and checkpoint DNA errors that lead to mitosis cancerous cells.

Thus, the application of DMBA in tumor initiation stage, chronic UVB irradiation in tumor promotion stage, was successful induced skin cancer in mouse skin model. In the future plan, we would like to determine skin cancer's markers in skin tissue for separation type of skin cancer including stage of evolutions.

Conclusion

DMBA/UVB-induced skin cancer on mice are mouse skin model that represent the multistage nature of skin cancer process in human. In addition, this model can be used to evaluate skin cancer prevention and the impact of genetic background on tumor initiation, promotion and progression stage. These findings supported a role of keratinocyte proliferation and epidermal hyperplasia in the evolution of skin cancer. Thus, this model appropriate to evaluate materials and prevention process in protected skin from carcinogens and UVB irradiation.

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