An UVB-carcinogenesis Model with KSN Nude Mice

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We established and characterized a systematic ultraviolet light-induced carcinogenesis model using KSN nude mice. We prepared five groups of KSN mice and exposed them six times a week to five levels of daily ultraviolet B (UVB) doses; 1340, 670, 320, 160 and 0 J/m²/day. In 670, 320 and 160 J/m²/day, the latency period tended to become shorter in proportion to the daily doses and prevalence data fitted well to log-normal distribution. In the log-log plot of days till 50% prevalence versus daily dose, we saw a linear relationship for 1mm tumor diameter. From this analysis, we determined that days necessary to reach 50% prevalence is in proportion to the −0.49 power of daily dose. The average number of tumors per survivor correlated with prevalence data. Direct measured rates of tumor growth were independent of daily UVB-dose. Therefore we speculated that UV-irradiation did not affect tumor growth after its appearance. Most UVB-induced tumors were squamous cell carcinoma, the rest were spindle cell carcinoma, papilloma and mixed type. We concluded that our experimental data with nude mice was in accordance with data with hairless mice in nature.

INTRODUCTION

Through the use of experimental animals to study the mechanism of UV-carcinogenesis, the carcinogenic effect of UV-irradiation has been well-described. Hairless mutant mice have been frequently used as a good experimental model for studying the nature of UV-carcinogenesis due to the reason of unnecessity of shaving. In addition to Blum’s early works¹⁻³, De Grujil et al.⁴ documented the wide range of dose-time dependency of tumor formation using hairless mice by

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chronic Ultraviolet B (UVB) exposure. In addition to these studies, an action spectrum, dose delivery effect and histology were analyzed with hairless mice5-7).

On the other hand, the immunological aspects of UV-carcinogenesis with mice have been also studied intensively because UV-induced skin tumor exhibited a very high degree of antigenicity. UV-induced tumors transplanted in syngeneic recipients were rejected, but they grew progressively in either immunodeficient or UV-irradiated recipients9). From this finding with immunocompetent mice, immunosuppressive effects of UV-irradiation was noted and an induction of suppressor T cells by UV-irradiation is assumed to be the essential phenomenon for UV-carcinogenesis9). However, it is unclear whether the immunological activity, except for macrophage10), contributes to immunosurveillance against UV-carcinogenesis or not. In addition to this, the contribution of immunological activity except for T-cells to the rejection of transplanted UV-induced tumor have not been studied yet. Even the role of T-cell activity in UV-carcinogenesis have been unclear until now11,12). We also have no direct evidence that other immunological activities such as natural killer (NK) suppresses UV-tumorigenesis in vivo even though the protective role of NK activity against neoplasm has been well recognized13-15). For analyzing the role of immunological activity against UV-carcinogenesis, we should compare UV-carcinogenesis among various immunodeficient animals under strict genetic control.

Before the comparison of UV-carcinogenesis among various immunodeficient mice, we must determine the experimental design such as daily dose etc. with parental KSN nude mice. However, few studies about UV-carcinogenesis with nude mice were reported until now. Though some researchers reported the experimental UV-induced skin tumorigenesis with immunodeficient athymic nude mice, they used single daily dose and tried only preliminary experiments12,16,17). Such reports were no use for our experiment then we decided to establish a new systematic UVB-carcinogenesis model with parental KSN nude mice in this study. Moreover our KSN mice are albino nude mice with prominent breeding performance and they are slightly larger than usual nude mice such as BALB/c-nu/nu mice18,19). We exposed them at five levels of UVB-irradiation and compared UVB-carcinogenesis with the reported data with hairless mice.

MATERIALS AND METHODS

Mice

KSN is an albino athymic nude mice strain that was established by Dr.K.Sudo10). All mice were maintained in compliance with the standards set forth in the “Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University”. Both breeding of and the irradiation of the mice by UV lamps were carried out in a specific pathogen free environment.

UVB-irradiation and Animal Observation

The UV source was six UVB lamps (Westinghouse FS-20, Westinghouse Electric Corporation, Pittsburgh, PA). These tubes provide a continuous UV spectrum with a peak at 313 nm. UV output was measured by a UV radiometer UV-103 (Macam Photometrics Limited, Livingston,
Scotland, UK) using 310 nm range. To avoid their intensity loss, the dose rate was checked every two months, and we kept it constant (3 × 10^2 µW/cm^2) during the experiment by changing the distance between mice and lamps.

Five week-old mice were used in the experiments, they were put into small cages on the turntable and exposed six times a week. The experimental design is shown in Table 1. Mice were macroscopically inspected once a week for tumor development.

In the present study we used the terms prevalence, tumor yield, time of 50% prevalence and tumor growth rate. The definition of these words are: prevalence means the percentage of mice with tumor that exceeded 1 mm diameter per number of survivors, tumor yield means average number of tumors per survivor, time of 50% prevalence means the time of UV-irradiation till 50% prevalence and tumor growth rate means a slope of the regression curve based on the plot of tumor weight with weeks of UVB-irradiation. When we plotted tumor weight against weeks, most suitable curve fitting was exponential formula; tumor weight = B × 10^A×week. In this formula both A and B indicate variables and A correlates to the growth rate of tumor. We then define this A value as the tumor growth rate. We euthanatized mice having more than 5 mm diameter tumor and we sampled tumor tissue. The mice that possessed more than 5 mm tumor were counted as survivor even after sampling. We followed “Batelle Columbus Laboratories Protocol” to calculate tumor weight. Tumor weight (mg) was described as L × W^2 × 1/2, where L and W are the width and length of tumors in mm.

**Histological Examination**

Tissue specimens from the tumors were fixed with Lillie solution and embedded in paraffin. In addition to the skin tumors, the same procedure was used for tissues from lungs, hearts, kidneys, livers and spleens. After staining with hematoxylin and eosin, we examined samples under a light microscopy.

**RESULTS**

*Tumor Development in KSN by UVB-irradiation*
We observed the backs of the mice once a week and sketched the back of every mouse. We judged tumor with more than 1 mm diameter as a "tumor" because smaller one than 1mm diameter was very difficult to detect macroscopically on nude mice. Prevalences as functions of time are plotted on a probability scale versus the logarithm of time in Fig. 1. The straight lines drawn in Fig. 1 are the best fits to the prevalence data using a log-normal distribution. Plotting in this way, points fall onto fitted straight line very well and this means UVB-carcinogenesis with KSN nude mice are log-normal distribution in nature. The latency periods of tumor induction were shorter in proportion to the daily doses. However, at the highest dose of 1340 J/m²/day, the latency period was longer than that at 670 J/m²/day in nude mice. The rate of tumor induction in 160 J/m²/day tended to be slower than other daily doses. There was no visible tumor on the dorsal skin of UV-unirradiated KSN mice during the experiment.

We plotted logarithm of the days till 50% prevalence (T50) determined from Fig. 1 versus daily dose (Fig. 2). From this plotting, we determined the relationship between the daily dose, except 1340 J/m²/day, and the days till 50% prevalence. The 50% prevalence is in proportion to -0.49 power of daily dose. Tumor yield as functions of time are plotted in Fig. 3. Only the first tumor of each mouse is taken into consideration in prevalence data. However, the tumor yield offers an alternative way of showing the carcinogenic effect of UV-irradiation. It is reasonable that observed tumor yield followed prevalence.

**Tumor Growth Rate**

To estimate the relationship between an average growth rate and daily dose, we measured the tumor diameter of more than eleven independent tumors per daily dose and calculated the tumor weight using "Battle Columbus Laboratories Protocol". We plotted tumor weight against weeks of UVB-irradiation and we showed the slope of the regression curve as tumor growth rate.
Fig. 4 shows average tumor growth rate of each daily dose and we detected daily dose-independence of tumor growth.

**Histological Analysis**

Almost all skin tumors available for histologic examination were classified as squamous cell carcinoma (Table 2). The rest were poorly differentiated squamous cell carcinoma (named spindle cell carcinoma), papilloma and mixed type. Squamous cell carcinoma was well-differen-
tiated and presented horn pearls. Spindle cell carcinoma lacked obvious keratinization, but the cellular morphology was similar to that in the epidermis. No metastasis to other organs was observed in all groups.

**DISCUSSION**

To our knowledge, though some researchers reported the experimental UV-induced skin tumor on athymic nude mice\textsuperscript{12,16,17}, no systematic experiments have been described until now. In this study, we established a systematic UVB-carcinogenesis model using KSN nude mice. This strain is albino, large and has no fur\textsuperscript{18,19} and these characteristics seem to be suitable for our
experiments. When we irradiated them with five levels of UVB-irradiation, their latency periods of tumor induction were shorter in proportion to the daily doses except for 1340 J/m²/day. Unexpectedly, the latency period in the 1340 J/m²/day irradiated group was longer than that in the 670 J/m²/day group in KSN mice as shown in Fig. 1. A satisfactory explanation for this effect has not been found in this study. However, we supposed that the reverse of dose response was due to tumor cell killing induced by high UVB dose because we sometimes observed the small ulcers nearby tumor. We then concluded that it is not practical to irradiate KSN mice at the daily doses exceeding 1340 J/m²/day and we decided to use the daily doses less than 670 J/m²/day in further experiments. Highest daily dose of De Gruijl et al. was 1900 J/m²/day and they observed no UV-induced ulcer. This discrepancy may due to the differences of the dosimetry system specially. Thermopile type dosimeter that De Gruijl et al. used can measure whole energy from UV-lamps and our Macam UV-103 can measure only its sensitive dose range from 270 nm to 370 nm. Then biological effectiveness of our highest daily dose, 1340 J/m²/day might be higher than 1900 J/m²/day in De Gruijl et al.. Other explanations can be offered are based on the inflammation response or the structural difference of skins between hairless and nude mice. On the other hand, the rate of tumor induction in 160 J/m²/day tended to be slower than those in other daily doses. This slow induction of tumors seemed to reflect the variation of each animal more clearly than those in other daily doses.

De Gruijl et al. studied the wide range of dose-time dependency of tumor formation on hairless mice exposed to chronic UVB exposure and they described mathematical analysis of UVB-carcinogenesis. Their result also supported a log-normal distribution of UVB-carcinogenesis in the same way with our result. However, when they determined the relationship between daily dose and the median appearance time of first tumor, they proposed the −0.6 power of time. Both our results as shown in Fig. 2 and Blum’s early work showed about −0.5 power of time. This discrepancy may be due to the different definition of tumor appearance. De Gruijl et al. defined the tumor appearance as the detection of a tumor with smaller diameter than 1 mm, but both we and Blum defined it as a tumor with more than 1 mm diameter. When De Gruijl used the definition similar to our condition, value between −0.5 and −0.6 was determined.

In our study (Fig. 4), direct measurement of tumor growth showed dose-independency in KSN mice and this result was in accordance with the result of De Gruijl et al. Then, we speculated that the growth of visible tumor of both immunocompetent and immunodeficient mice is independent of subsequent UV-irradiation. Blum’s hypothesis of UV-accelerated tumor growth was at variance with the experimental results of not only hairless mice but also immunodeficient mice.

Histologically, most prominent tumors were squamous cell carcinoma as shown in Table 2. When compared with other reports on UV-induced tumors in nude mice, there were no major histological differences. Furthermore, De Gruijl et al. reported that almost all tumors originated from epidermis in their UVB-irradiated hairless mice. Taking into account these results, we can conclude that the target tissues of UVB-carcinogenesis in mice is the epidermal layer, and there is no outstanding difference between others and our immunodeficient mice in UVB-carcinogenesis.

In conclusion, UVB-carcinogenesis in KSN nude mice is similar to that of hairless mice in
both tumor induction and histological analysis. An introduction of immunodeficiency to mice do not affect the nature of UVB-carcinogenesis examined so far.

Since we have already established various strains of immunodeficient mice with KSN's genetic background\(^1\), we can now compare the carcinogenic effect of UV-irradiation under various immunodeficient conditions and we can analyze the contribution of immunological activity to UVB-carcinogenesis using these strains.

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