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Skin Tumor Susceptibility to 7, 12-Dimethylbenz[*a*]anthracene in Transgenic Rats Carrying Human c-Ha-ras Proto-oncogene

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We have established a transgenic rat line carrying three copies of the human c-Ha-ras proto-oncogene with its own original promoter region, Jcl/SD-TgN (Hras gen) 128Ncc rat. These rats are highly susceptible to *N*-methyl-*N*-nitrosourea induced mammary carcinogenesis. To examine their susceptibility to 7, 12-dimethylbenz[*a*]anthracene (DMBA) induced skin carcinogenesis, male transgenic rats and wild-type littermates were treated with a single dose of DMBA. Many tumors were observed only in the DMBA treated Hras-128 transgenic rats, but not in wild type. These results indicate that transgenic rats carrying human c-Ha-ras proto-oncogene are highly susceptible to DMBA-induced skin carcinogenesis and may be utilized as a rat model for analysis of skin tumorigenesis.

Key Words: Skin carcinogenesis, Transgenic rats, c-Ha-ras oncogene, DMBA, TPA

INTRODUCTION

The carcinogenicity tests are indispensable when evaluating the safety of drugs in the process of newly developed and when identifying environment carcinogens as well. In order to develop rapid carcinogenecity testing system, animals which are susceptible to carcinogens are indispensable. Transgenic mice provide us with good animal models for many disease and widely used for analysis of various gene function. Recently, studies on the validation for the use of either p53 knockout mice,[1)] v-Ha-ras transgenic mice,[1)] c-Ha-ras transgenic mice,[2)] pim-1 transgenic mice[3)] as short term bioassay models for identifying chemical carcinogens have been reported. For studies of chemical carcinogenes, however, rats are more frequently used for several reasons. First, there are no equivalent markers lesion for mouse liver. Second, many virus effect the mouse mammary carcinogenesis. Now only limited types of transgenic rats have been developed

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for study carcinogenesis. We have generated transgenic rats (named H-ras 128) using the same gene used for generation of human c-Ha-ras proto-oncogene transgenic mice.[4] Also, we showed that Hras 128 transgenic rats are highly susceptible to *N*-methyl-*N*-nitrosamine (MNU)-induced mammary carcinogenesis[5] and *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) induced bladder carcinogenesis.[6]

The two stage skin carcinogenesis concept was introduced into experimental tumor research by Berenblum[7] and Mottram[8] in the early forties. Since then the two-stage experiment has virtually been confined to the dorsal epithelium of mouse because early attempts by Shubik[9] to repeat the experiment in the rat, guinea-pig and rabbit were unsuccessful and seemed to indicate a strong species-and organ-specificity of the initiator-promoter combination DMBA and 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). Other research confirmed that skin carcinogenesis was weak after the low DMBA doses (2.5 mg).[10] On the contrary, Schweizer *et al.*[11] showed that both combinations DMBA-TPA, and ethyldiazoacetate-TPA led to a high incidence of epithelial tumors, although ethyldiazoacetate seems to be a suitable alternative to DMBA for systemic initiation of skin tumors.

In this study, we investigated the skin carcinogenesis of H-ras 128 transgenic rats carrying human prototype c-Ha-ras gene to DMBA-TPA and compared it with that of control-wild type rats.

MATERIALS AND METHODS

1) Transgenic animals

Sprague-Dawley rats (CLEA Japan, Inc., Tokyo, Japan) were used for the initial production of founder animals of transgenic rats. Techniques used for generation of transgenic rats were essentially similar to those commonly used for transgenic mice.[4] The F_1 offspring were screened by the polymerase chain reaction or Southern blot analysis for the presence of the human proto-type c-Ha-ras gene.

2) Treatments

Seventy-four 5-week-old Hras 128 transgenic rats and Non-transgenic rats were used in the experiments. The dorsal area of the rats was shaved 3 day prior to the experiment[11] and the animals were assigned by random distribution each 4 experimental groups. Group1 (Control group): The animals received topical applications of 0.5 ml acetone 3 times per week for 20 weeks. Group 2 (TPA group): The animals were topically treated 3 times per week for 20 weeks with 3.05×10^{-5} mg TPA in 0.5 ml acetone. Group 3 (DMBA group): The animals were initiated by a single topical application of 2.5 mg DMBA in 1 ml acetone. Group 4 (DMBA-TPA group): The animals were initiated according to group 3, two weeks later they were promoted as indicated for group 2. DMBA and TPA were from Sigma Chemical co., USA. All substances were administrated between 9.00 and 10.00 a.m. over the whole period of treatment. During the experiment shaving was routinely performed once per week. Skin tumors were recorded weekly. All animals used were handled in accordance with guidelines an animal experimentation of each institute. The incidence and multiplicity of proliferative lesions were analysed by Fisher's exact probability test and Student's *t*-test, respectively.

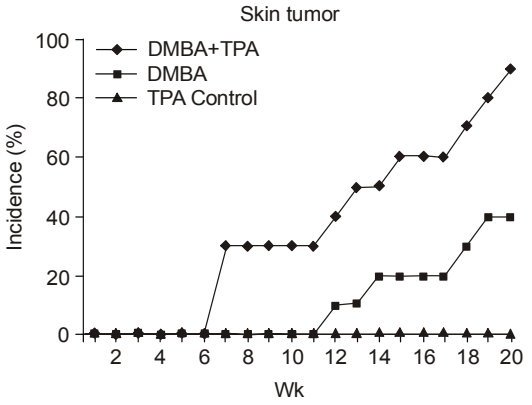

Fig. 1. Periodical observation of skin tumors in c-Ha-ras transgenic rats treated with DMBA and TPA at 20 week.

RESULTS

DMBA is known to produce squamous cell carcinoma of the skin and mammary carcinoma of mammary gland. Fig. 1 shows the overall skin tumor rate in Groups 3 (DMBA) and 4 (DMBA-TPA), beginning from week 7 after promotion up to the week 20. Control animals and Non-transgenic animals were free of tumors over the whole period of observation. In contrast, after 7 weeks promotion, 30% of the Hras 128 transgenic animals in group 4 had already developed tumors (Fig. 1). As indicated in Table I, fourteen out of 15 (93%) DMBA-TPA treated male Hras 128 transgenic rats developed skin tumors of the skin and 90% of DMBA-treated transgenic rats bearing skin tumors 20 week after DMBA initiation (Fig. 2). The size of papilloma were 22.7±14.5 and 31.9±27.6 mm in Group 3 and Group 4 respectively. Number of papilloma were 3.63±2.13 and 6.07±4.5 in Groups 3 and 4, respectively. No skin tumors were observed in DMBA-TPA-treated non-transgenic rats or in acetone treated control animals. Histologically different tumor type such as papilloma, fibroadenoma,

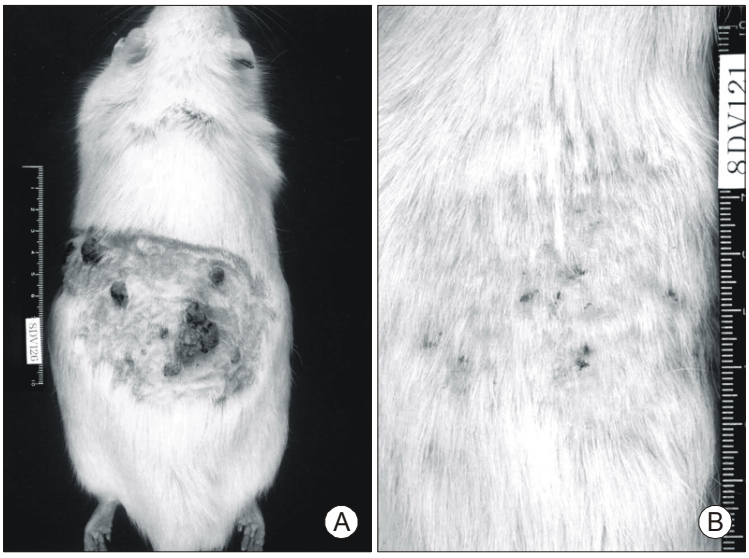

Fig. 2. Macroscopic appearance of the dorsal area of animals in group 4. (A) transgenic rat, (B) wild type rat.

Table 1. Quantitative data for skin tumors induced DMBA and TPA in c-Ha-ras transgenic rats

Type	Treatment	Incidence	No. of Papilloma/rat	Size of Papilloma[a] (mm)
Hras-128	DMBA+TPA	14/15 (93.3%)[b]	6.07±4.5[b]	31.9±27.6[b]
Wild	DMBA+TPA	0/15	0	0
Hras-128	DMBA	9/10 (90%)[b]	3.63±2.13[b]	22.7±14.5[b]
Wild	DMBA	0/10	0	0

[a]: Maximum diameter, [b]: Significantly different from corresponding non-Tg rats at $p < 0.0001$

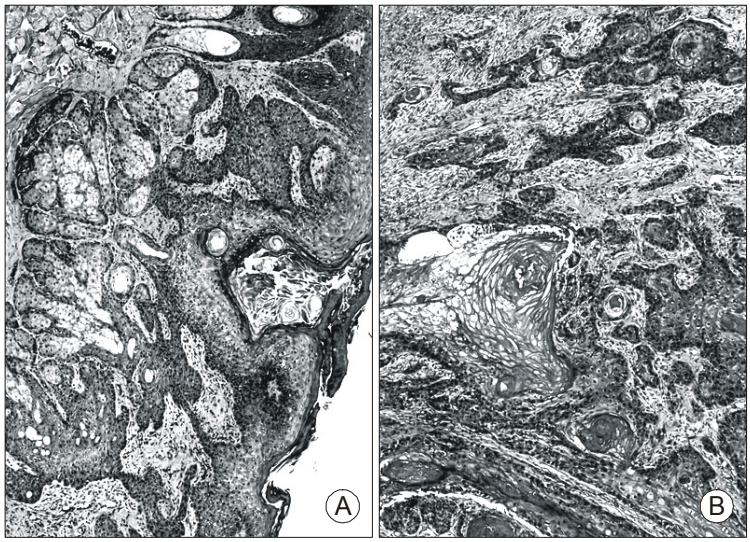

Fig. 3. Representative histological appearance of skin tumors induced in c-Ha-ras transgenic rats by DMBA and TPA (A) papilloma, (B) squamous cell carcinoma. H&E ×100.

observed in squamous cell carcinoma bearing transgenic rats (Fig. 3).

DISCUSSION

The present study revealed that the transgenic rats carrying three copies of the human c-Ha-ras proto-oncogene, the same gene as used to established transgenic mice, responded to a single painting of DMBA 2.5 mg in 1ml acetone by developing multiple, large sized skin tumors. The earliest visible tumors appeared at week 7, rapidly increasing their size and number up to 6 tumors per rat. The results clearly indicate that expression of the transgene is associated with marked enhancement of susceptibility to DMBA skin carcinogenesis. Our experiment clearly showed that wild type SD-rat resist to DMBA-TPA mediated tumor induction. On the while, only DMBA treatment induced tumor incidence, which means that transgene may be promoter for skin carcinogenesis procedure.

Cellular proto-oncogene can be activated by both point mutation and chromosomal translocation.[12] So many tumors in mice initiated by DMBA have a specific A-T transverse at the second nucleotide of codon 61 of the Harvey-ras (Ha-ras) gene.[13] Transgenic mice prepared using the same DNA construct of human c-ha-ras proto-oncogene were reported to have high susceptibility to DMBA induction of forestomach, skin and lung tumors in which mutations of the transduced human c-Ha-ras proto-oncogene at codon 61 CAG to CTG were frequently observed.[14] Furthermore, no mutations in their endogenous mice c-Ha-ras gene were detected, clearly indicating that the transgene is a target of carcinogens.[14] However, Asamoto *et al.*[5] showed that rats carrying transduced human c-Ha-ras gene are highly susceptible to MNU-induced mammary tumor and that this is not primarily due to mutations of the transgene or endogenous c-Ha-ras gene. In conclusion, human c-Ha-ras proto-oncogene transgenic rats reported show remarkable enhancement in the susceptibility to DMBA-skin carcinogenesis, these rats could be utilized for the short-term detection of carcinogens and promoting agents in the skin.

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사람 c-Ha-ras Proto-oncogene이 주입된 형질전환 랫드에서 12-Dimethylbenz[*a*]anthracene에 의한 피부발암 감수성 평가

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사람 c-Ha-ras 발암유전자를 주입한 랫드를 생산하여 피부발암 감수성을 알아보고자 하였다. 5주령의 형질전환 랫드에 DMBA (2.5 mg/ml, 1회)를 처치한 후 TPA (100 nM, 3회/주)를 20주까지 도포 한 뒤 피부의 육안적인 변화를 기록하고, 조직을 처리하여 관찰하였다. 형질전환 랫드의 도포된 피부에서 선암과 편평세포암이 발생하였으며, 대조군에서는 이러한 병변이 관찰되지 않았다. 이러한 결과로부터 이 형질전환 랫드는 피부발암 감수성이 매우 높으며 피부암발생의 기전연구 및 암예방물질을 실험하기에 좋은 모델동물로서 이용가치 있다고 생각한다.

Key Words: 피부 발암, 형질전환 랫드, c-Ha-ras 발암유전자, DMBA, TPA