RAPID COMMUNICATION Jpn. J. Cancer Res. 87, 1097–1101, November 1996

Genetic Controls of Susceptibility and Resistance to 4-Nitroquinoline 1-Oxide-induced Tongue Carcinomas in Rats

Motoo Kitano,[1] Yoshikazu Hirayama,[1] Jun-ichi Tanuma,[1,6] Hiroaki Matsuuchi,[1] Yoshihiro Miura,[1] Tie-Jun Li,[1] Ichiro Semba,[1] Hiroki S. Ozaki,[2] Teiji Kokubu,[3] Hiromichi Hatano,[4] Mariko Tada,[5] Yasuto Kobayashi[6] and Hayase Shisa[6]

[1]Department of Oral Pathology and [2]Department of Oral Anatomy, Kagoshima University Dental School, 8-35-1 Sakuragaoka, Kagoshima 890, [3]Department of Agricultural Sciences and Natural Resources, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890, [4]School of Nursing, Faculty of Medicine, Kurume University, 777-1 Higashi-Kushihara-machi, Kurume 830, [5]Department of Biochemistry, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chigusa-ku, Nagoya 464 and [6]Department of Pathology, Saitama Cancer Center Research Institute, 818 Komuro, Ina, Kitaadachi-gun, Saitama 362

We analyzed the incidence of infiltrative mass-type tongue carcinomas (IMTC) induced in 550 rats by continuous oral administration of 0.001% 4-nitroquinoline 1-oxide solution for 180 days. The study included various crosses of susceptible Dark-Agouti rats (DA) and resistant Wistar/Furth rats (WF). DA showed a 93.6% incidence of IMTC measuring more than 5 mm in their largest diameter, while WF showed only a 4% incidence. Reciprocal F_1 and F_2 hybrids mated by DA and WF showed 47.5% and 45.8% incidences, respectively. Meanwhile, reciprocal backcrossed hybrids to DA and WF showed 73.7%, and 24.6% incidences, respectively. Segregation of the incidences suggests that there are two autosomal dominant genes, one linked to the susceptibility of DA and the other to the resistance of WF.

Key words: 4-Nitroquinoline 1-oxide — Tongue carcinoma — Susceptibility — Resistance — Genetic control

Although the induction of squamous cell carcinomas of the tongue by long-term treatment with 4-nitroquinoline 1-oxide (4NQO) is a commonly observed phenomenon in rats, susceptibility and resistance are highly strain-dependent. Previously, we showed that Dark-Agouti rats (DA) were highly susceptible to the development of tongue carcinomas after continuous oral application of 4NQO. In contrast, Wistar/Furth rats (WF) were much more resistant to tongue carcinogenesis than were DA.[1)] These results suggested the existence of genes regulating susceptibility and resistance to 4NQO-induced tongue carcinoma in rats. In the present study, to investigate the inheritance of susceptibility and resistance to 4NQO-induced tongue carcinoma, a number of crosses were made involving highly susceptible DA and highly resistant WF, and the incidences of tongue carcinomas in the parental strains and crosses were observed.

The rats (total, 550) used in this study were as follows. 1) Inbred DA (DA/Sls) from Shizuoka Laboratory Animal Center, Hamamatsu. 2) WF, which had been introduced in 1976 from Hiroshima University, Hiroshima to our laboratories. They were at the 78th to 82nd generation of sister-brother mating. 3) The F_1 hybrids of the DA and WF were obtained by reciprocal mating between these two strains (F_1-a: DA×WF, F_1-b: WF×DA). 4) The F_2 hybrids, F_2-a and F_2-b, were obtained from crosses between the females and males of F_1-a and F_1-b, respectively. 5) Reciprocal backcrosses to DA and WF of F_1-a and F_1-b, i.e., DA×F_1-a, F_1-a×DA, DA×F_1-b, F_1-b×DA, WF×F_1-a, F_1-a×WF, WF×F_1-b and F_1-b×WF, were also obtained. The rats were weaned three weeks after birth and housed in plastic cages in an air-conditioned room at 22°C±2°C. They were fed a commercial pelleted CE-2 diet (Japanese CLEA Co., Tokyo) and given tap water *ad libitum*. The number and groups of rats used in this experiment are shown in Table I.

4NQO (Nakalai Tesque, Kyoto) was dissolved in 5% ethanol solution at a concentration of 200 mg/liter to obtain a stock solution, which was kept refrigerated. Immediately before use, the stock solution was dissolved in distilled water to make a final concentration of 0.001% 4NQO. All the rats, at the age of six weeks, were allowed access to drinking water containing 0.001% 4NQO *ad libitum* from 5 p.m. to 9 a.m., but given no water thereafter. The rats were inspected twice a day and weighed once a week. The volume of NQO-containing water consumed was measured daily to estimate the intake of 4NQO; this was approximately 70 mg/100 g body weight/day for each rat with no significant differ-

Table I. Observed Incidences of Rats with Infiltrative Mass-type Tongue Carcinomas (IMTC) and Predicted Genotypes with Expected Incidences of Phenotypes in our Two-gene Model

Groups of experimental rats	Observed incidences of IMTC in experimental rats (%)		Predicted genotypes and expected incidences of phenotypes						
			Predicted genotypes[a]				Incidences of genotypes	Expected incidences of phenotypes[b] (%)	Overall expected incidences of phenotypes[c] (%)
Dark-Agouti (DA)	93.6	(44/47)[d]	*STC*	*STC*	*rtc*	*rtc*	1/1	100	100
Wistar/Furth (WF)	4	(2/50)	*stc*	*stc*	*RTC*	*RTC*	1/1	0	0
F_1[e]	47.5	(28/59)	*STC*	*stc*	*RTC*	*rtc*	1/1	50	50
F_2[e]	45.8	(65/142)	*STC*	—	*rtc*	*rtc*	3/16	100	46.9
			STC	—	*RTC*	—	9/16	50	
			stc	*stc*	*RTC*	—	3/16	0	
			stc	*stc*	*rtc*	*rtc*	1/16	0	
Backcross to DA[f]	73.3	(84/114)	*STC*	—	*rtc*	*rtc*	2/4	100	75
			STC	—	*RTC*	*rtc*	2/4	50	
Backcross to WF[f]	24.6	(34/138)	*STC*	*stc*	*RTC*	—	2/4	50	25
			stc	*stc*	*RTC*	—	2/4	0	
Total	46.7	(257/550)							

a) The details of *STC, stc, RTC* and *rtc* are described in the text.
b) See the text for details.
c) Expected values of the incidences of IMTC estimated from the predicted genotypes.
d) Numbers of rats with IMTC/numbers of experimental rats.
e) F_1 includes reciprocal hybrids of DA and WF and F_2 includes two kinds of hybrids.
f) Reciprocally backcrossed hybrids are included.

Fig. 1. A large, infiltrative mass-type tongue carcinoma of a rat of the Dark-Agouti strain (No. male-12) that died on the 161st experimental day.

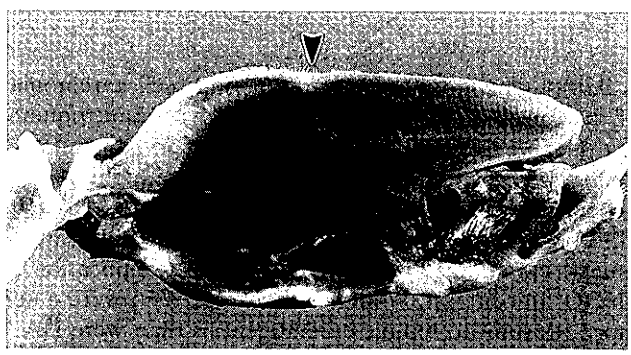

Fig. 2. A very small carcinoma (arrow) in the tongue of a rat (No. female-6) of the Wistar/Furth strain killed on the 180th experimental day.

ences between sexes, strains or hybrids. About 10 rats of both sexes in each group were used as controls and 4NQO was not administered. The experimental animals were killed when they became moribund, or on the 180th day of the experiment, if they were still alive. A full autopsy with histopathological examination of the various organs (brain, heart, lungs, liver, spleen, pancreas, kidneys, adrenals, testicles, ovaries and digestive tract from the lip to rectum, including the tongue, maxilla and mandible) was carried out on each animal.

The differences in the incidences of infiltrative mass-type tongue carcinomas (IMTC) between the experimental groups was evaluated using the χ^2 test. In this paper, the term 'IMTC' is used for carcinomas with a largest diameter of 5 mm (or more) showing apparent infiltrative growth into the tongue muscles.

Tongue carcinomas were found to develop more frequently in DA than in WF (Table I), confirming our previous findings.[1] Most of the examined DA had a survival time of less than 180 days and only 9 of the 47

DA rats (19%) survived past the 180th day. The mean observation period was 160.9 days (SD, 18.85), ranging from 117 to 180 days. Most DA showed development of one or more IMTC (Fig. 1), and the incidence of IMTC in DA was 93.6%. Only three rats had multiple tongue carcinomas where the diameters did not reach 5 mm. All the examined DA had multiple carcinomas in various sites of the oral cavity other than the tongue; some of these carcinomas were large enough to be categorized as IMTC.

All of the 50 WF were alive and appeared healthy on the 180th experimental day. Two of them (4%) had an IMTC of the tongue. Twenty-six had one or two small tongue carcinomas with diameter < 5 mm (Fig. 2). The remaining 22 animals showed slight hyperkeratosis with more or less marked dysplastic changes (but not frank malignancy) on the surface epithelium of the tongue. Two WF did not have any carcinomas in any part of the body and the remaining 48 animals had carcinomas in various sites of the oral cavity other than the tongue, though most of them were very small.

The F_1 generation produced by mating the female DA with the male WF (F_1-a) and the female WF with the male DA (F_1-b) had similar incidences of IMTC, 51.6% (16/31) and 42.9% (12/28), respectively, with an overall incidence of 47.5%. Comparison of the two reciprocal groups of F_1 rats did not show any difference in the incidence of carcinomas between sexes. The pooled data of 142 F_2 hybrids including both F_2-a rats and F_2-b rats showed an incidence of 45.8% (65/142) without apparent differences between the sexes or the reciprocal progeny groups.

Subsequently, the F_1 hybrids were backcrossed reciprocally to DA and WF and the progeny was given 4NQO. In this experiment, four combinations of backcrosses to DA and WF were used; the pooled data are shown in Table I. The backcrosses to DA showed an incidence of 73.5% (84/114) of IMTC while the backcrosses to WF showed an incidence of 24.6% (34/138).

The F_1 rats survived longer than DA and 75% of the F_1 rats were still alive at the 180th experimental day; their observation period ranged from 157 to 180 days with a mean value of 177.4 days (SD, 5.76). As for the F_2 rats, 75% survived to the last experimental day and the mean observation period was 175.8 days (SD, 9.90), ranging from 129 to 180 days. Approximately 64% of the backcross hybrid to DA and 91% of the backcross hybrid to WF survived beyond the 180th experimental day and the observation periods ranged from 117 to 180 days and from 150 to 180 days, respectively [mean: 171.8 days (SD, 14.81) and 178.9 days (SD, 4.20)]. Sex differences in the length of the observation periods were not identified among any of the experimental groups ($P > 0.05$).

The coat color of DA is agouti non-hooded without Irish patches and that of WF is albino. The F_1 rats are agouti non-hooded with Irish patches. Coat color phenotypes in the F_2 animals are segregated into agouti non-hooded with or without Irish patches, black non-hooded with or without Irish patches, agouti hooded, black hooded and albino. We could not find any apparent relationships between the coat color phenotypes and the susceptibility to 4NQO-induced carcinogenesis (data not shown).

With chemically induced carcinogenesis, host genetic controls or influences have been identified in, for example, activation or inactivation of the metabolism of carcinogens, pathophysiological characteristics of the target organs and tissues, activity for repair of DNA injuries of the target cells and immunity to transformed cells. There

Table II. Some One-gene Models of Infiltrative Mass-type Tongue Carcinomas (IMTC) of the Rat

Groups of experimental rats[a)]	Predicted genotypes[b)] and their incidences			Expected incidences of IMTC of the tongue (%)[c)]	Observed incidences of IMTC of the tongue (%)
Dark-Agouti (DA)	*STC*	*STC*	1/1	100	93.6
Wistar/Furth (WF)	*stc*	*stc*	1/1	0	4
F_1	*STC*	*stc*	1/1	100	47.5
F_2	*STC*	—	3/4	75	45.8
	stc	*stc*	1/4		
Backcross to DA	*STC*	—	2/2	100	73.3
Backcross to WF	*STC*	*stc*	1/2	50	24.6
	stc	*stc*	1/2		

a) The experimental rats are the same as those of Table I.
b) STC is an autosomal dominant gene providing susceptibility to 4NQO-induced tongue carcinomas, and *stc* is a recessive allelic gene, which is non- or hypofunctional as regards the susceptibility.
c) Expected values of the incidences of IMTC estimated from the predicted genotypes.

is evidence that 4NQO can be converted *in vivo* to a highly reactive metabolite, 4-hydroxyaminoquinoline 1-oxide.[2-4] This metabolite formed in target tissues may act as a proximate carcinogen by covalently binding to nuclear DNA. Covalent modification of DNA has been proposed as a general mechanism for carcinogenesis by many compounds. However, glutathione transferases can conjugate intracytoplasmic glutathione with 4NQO, and the conjugate is no longer carcinogenic.[4-6] In spite of this, little is known at present about the genetic differences in metabolic fate and tissue distribution of 4NQO in rats.

Based upon our present results, together with the previous finding that the proliferative response of DA tongue epithelium to 4NQO stimulation was much higher than that of WF tongue epithelium,[1] we postulate a two-gene model (Table I) to explain the susceptibility and resistance to 4NQO-induced tongue carcinogenicity in rats. As illustrated Table I, an autosomal dominant gene, *STC,* codes for a tongue mucosal epithelium protein, which is essential for the activation of epithelial proliferation under stimulation by 4NQO. DA are homozygous for *STC,* while WF carry a nonfunctional or hypofunctional recessive gene *stc* at this locus. Conversely, WF have another autosomal gene, *RTC,* which endows them with resistance to cellular proliferation. With respect to the locus for *RTC,* DA is homozygous for the recessive gene *rtc,* which is nonfunctional or hypofunctional for resistance to cellular proliferation. Hybrid rats with the allele of *STC- RTC-,* in which *STC*- and *RTC*-proteins will be made concurrently, are expected to have a 50% probability of developing a tongue carcinoma large enough to allow classification of the animals as tumor susceptible by our criteria. For the parental DA, parental WF, F_1, F_2 and the backcrosses to DA as well as to WF, tumor incidences predicted by this model are 100%, 0%, 50%, 46.9%, 75% and 25%, respectively, which are in good agreement with the experimentally determined incidences. The adequacy of the model in accounting for the experimental data was tested by using χ^2 analysis, and the test did not reject the model. Similar models involving one genetic locus (Table II) were also evaluated statistically, but they did not fit our experimental data well.

Although, in general, the relationship between genetic factors and susceptibility and/or resistance to carcinogenicity is highly complex, in some cases simple gene models have been proposed which can adequately account for the appearance of specific types of cancer.[7-16] Our data suggests that such a situation also exists in DA and WF.

We are indebted to Professor Hiroshi Hiai, Department of Pathology and Biology of Diseases, Kyoto University Graduate School of Medicine for his critical review of this study and valuable comments. We thank Misses Fusako Kataoka and Kyoko Machida, Mr. Shigeto Fukushige and Mrs. Akiko Yanagida for their technical assistance. This manuscript was kindly revised by Drs. Gabriel Landini and Richard M. Shelton, School of Dentistry, Birmingham University (UK).

(Received June 14, 1996/Accepted August 21, 1996)

REFERENCES

1) Kitano, M., Hatano, H. and Shisa, H. Strain difference of susceptibility to 4-nitroquinoline 1-oxide-induced tongue carcinoma in rats. *Jpn. J. Cancer Res.*, **83**, 843–850 (1992).
2) Sugimura, T., Okabe, K. and Endo, H. The metabolism of 4-nitroquinoline 1-oxide to 4-hydroxyaminoquinoline 1-oxide in rat liver and hepatomas. *Cancer Res.*, **26**, 1717–1721 (1966).
3) Kawazoe, Y., Tamura, M. and Araki, M. Metabolism of carcinogenic 4-hydroxyaminoquinoline 1-oxide in mice. *Gann*, **61**, 593–596 (1970).
4) Stanley, J. S., York, J. L. and Benson, A. M. Nitroreductases and glutathione transferases that act on 4-nitroquinoline 1-oxide and their differential induction by butylated hydroxyanisole in mice. *Cancer Res.*, **52**, 58–63 (1992).
5) Stanley, J. S., Lay, J. O., Jr., Miller, D. W. and DeLuca, C. Identification of the glutathione conjugate of 4-nitroquinoline 1-oxide formed in the reaction catalyzed by murine glutathione transferases. *Carcinogenesis*, **10**, 587–591 (1989).
6) Aceto, A., Di Ilio, C., Lo Bello, M., Bucciarelli, T., Angelucci, S. and Federici, G. Differential activity of human, rat, mouse and bacteria glutathione transferase isoenzymes towards 4-nitroquinoline 1-oxide. *Carcinogenesis*, **11**, 2267–2269 (1990).
7) Evans, J. T., Shows, T. B., Sproul, E. E., Paolini, N. S. and Mettelman, A. Genetics of colon carcinogenesis in mice treated with 1,2-dimethylhydrazine. *Cancer Res.*, **37**, 134–136 (1977).
8) Wiklund, J., Rutledge, J. and Gorski, J. A genetic model for the inheritance of pituitary tumor susceptibility in F344 rats. *Endocrinology*, **109**, 1708–1714 (1981).
9) Ohgaki, H., Kawachi, T., Matsukura, N., Morino, K., Miyamoto, M. and Sugimura, T. Genetic control of susceptibility of rats to gastric carcinoma. *Cancer Res.*, **43**, 3663–3667 (1983).
10) Naito, M., Ito, A. and Aoyama, H. Genetics of susceptibility of rats to trigeminal schwannomas induced by neonatal administration of *N*-ethyl-*N*-nitrosourea. *J. Natl. Cancer Inst.*, **74**, 241–245 (1985).
11) Shisa, H. and Hiai, H. Genetically determined susceptibil-

ity of Fischer 344 rats to propylnitrosourea-induced thymic lymphomas. *Cancer Res.*, **45**, 1483–1487 (1985).
12) Gould, M. N. Inheritance and site of expression of genes controlling susceptibility to mammary cancer in an inbred rat model. *Cancer Res.*, **46**, 1199–1202 (1986).
13) Ohgaki, H., Tomihara, N., Sato, S., Kleihues, P. and Sugimura, T. Differential proliferative response of gastric mucosa during carcinogenesis induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in susceptible ACI rats, resistant Buffalo rats, and their hybrid F_1 cross. *Cancer Res.*, **48**, 5275–5279 (1988).
14) Isaacs, J. T. Inheritance of a genetic factor from the Copenhagen rat and the suppression of chemically induced mammary adenocarcinogenesis. *Cancer Res.*, **48**, 2204–2213 (1988).
15) Mock, B. A., Krall, M. K. and Dosik, J. K. Genetic mapping of tumor susceptibility genes involved in mouse plasmacytomagenesis. *Proc. Natl. Acad. Sci. USA*, **90**, 9499–9503 (1993).
16) Yamada, Y., Shisa, H., Matsushiro, H., Kamoto, T., Kobayashi, Y., Kawarai, A., Hiai, H. T lymphomagenesis is determined by a dominant host gene *thymic lymphoma susceptible mouse-1 (TLSM-1)* in mouse models. *J. Exp. Med.*, **180**, 2155–2162 (1994).