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Allelic Imbalances on Chromosome 20 in Human Transitional Cell Carcinoma

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One determinant of the survival time of cancer-bearing patients may be genetic factors. In chemically induced bladder cancers of mice, differences in survival time have been observed among several inbred strains. Genetic analyses of such differences in crosses between C57BL/6 and NON mice revealed that the survival period is determined by two quantitative trait loci on mouse chromosomes 6 and 2, respectively. We explored the possibility that genetic alterations may be observed in the syntenic conserved chromosomal regions of human transitional cell carcinoma corresponding to mouse chromosomes 6 and 2. Human chromosome 7, containing a region syntenic to mouse chromosome 6, is reported to harbor frequent genetic alterations in bladder cancers. In this study, we investigated 70 human urothelial cancers for possible genetic alterations on human chromosome 20p and 20q containing regions syntenic to mouse chromosome 2. Allelic imbalances were observed in 22 cases (31.4%) on 20p and 18 cases (25.7%) on 20q. Those allelic imbalances, however, did not show a direct correlation with the prognosis of the patients. Higher grade tumors tended to show more frequent imbalances on chromosome 20; however, this tendency was not significant.

Key words: Urinary tract — Loss of heterozygosity — Chromosome 20

Genetic susceptibility to cancer is widely accepted to be a polygenic trait. Carcinogenesis is a complex process involving a number of host and environmental factors. Genetic loci determining cancer susceptibility have been largely obscure, except for several loci relevant to some familial cancers, because the effects of most of these loci are quantitative in nature and represent the sum of many polymorphic gene actions, rather than simple loss or unusual gain of a single gene function. In several animal models, increasing evidence of genetic differences in cancer susceptibility among strains has been accumulated. Some of the steps under such gene control may be specific to the species or method of cancer induction, but others may well be commonly shared among various species. There are difficulties in discovering human cancer susceptibility genes, but investigation of homologous genes in human cancers may be aided when a genetically controlled step is identified in an animal model. Synteny conservation among species and increasing precision of comparative genetic maps may favor such an approach.

There have been a number of studies on genetic alterations in human bladder cancers. Loss of heterozygosity (LOH) at loci on chromosome (Chr.) 9p and/or 9q is the most frequent genetic alteration in transitional cell carcinomas (TCC) of the bladder.[1)] Furthermore Chrs. 4, 8, 10, 11, 13, 14 and 17 have also been reported to bear high frequencies of LOH.[2–4)] Using a mouse chemical-induced bladder cancer model, we recently reported two loci affecting the survival period of carcinogen-treated mice.[5)] One of these loci has been mapped on Chr. 6, in a region syntenic to the long arm of human Chr. 7. The oncogenes *met* and *pleiotrophin*, which have been reported to be associated with the prognosis or with invasiveness of human bladder cancer,[6,7)] are mapped in this region. On the long arm of human Chr. 7, LOH has been reported to occur in 34–59% of cancers of several organs, i.e., breast, prostate, ovary and myeloid neoplasms.[8–11)] Using the above-mentioned mouse bladder cancer model, we identified another locus on 89 cM of mouse Chr. 2.[5)] The region containing this locus is homologous to the human Chr. 20p and 20q arms. According to the Chromosome Committee reports for the mouse genome,[12)] mouse Chr. 2 consists of a number of regions syntenic to different human Chrs. However, the genes on human Chr. 20, for which mouse homologues are detected, are all mapped in a region 73.2–110 cM from the centromere of mouse Chr. 2. This region contains exclusively the syntenic region of human Chr. 20, and no other. There have been few studies on LOH in human bladder cancers with respect to human Chr. 20. In this study, we examined genetic alterations on Chr. 20 in human urothelial cancers and found allelic imbalances distributed on both the short and long arms.

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MATERIALS AND METHODS

Samples and DNA extraction Tumor and peripheral blood samples of 70 patients with TCC of the urinary tract were used. The patients were surgically treated at Kyoto University Hospital. Tumor specimens were obtained by total cystectomy, nephroureterectomy or transurethral resection (TUR) of bladder tumors, and frozen immediately at −80°C. DNA was extracted from the tissues according to the methods described previously.[13] Histopathological diagnoses were based on the UICC classification.[14] Other prognostic parameters were determined at the time of obtaining the specimens.

PCR and detection of DNA disparity We chose 11 microsatellite marker loci along Chr. 20: six on the 20p and five on the 20q arms. The map positions were from the Marshfield sex averaged map of human Chr. 20 and CEPH/Genethon Chromosome 20 Linkage Map. The 5′ end of each forward primer was labeled with 6-carboxyfluorescein dye. PCR with fluorescence-tagged markers was carried out in a volume of 10 μl containing 50 ng of template DNA, each dNTP at 21.25 μM, 0.33 μM primer, GeneAmp PCR buffer and 0.5 units of AmpliTaq (Perkin Elmer Applied Biosystems, Foster City, CA). The thermal profile was as follows: 94°C for 2 min; 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; and a final extension of 72°C for 10 min in a PC-800 thermal cycler (ASTEC Co., Fukuoka). Products and TAMRA500 size standard (Perkin Elmer Applied Biosystems) were loaded on 5% polyacrylamide 6 *M* urea 36-cm-long gels and electrophoresed in an ABI377 auto-sequencer. Scanned images were analyzed with GeneScan software (Perkin Elmer Applied Biosystems) to acquire quantitative allele signal strength data. In the case of constitutional heterozygotes, two alleles were detected in normal tissue, and if the ratio of the two alleles was significantly changed in the tumor, single allele deletion or gain was suspected. When the size of the allele in the tumor differed from that in normal tissue, we considered it to reflect microsatellite instability. We checked for allelic imbalance by calculating the ratio of the integral intensity.[15] A sample generates one quotient from the intensity of two alleles. The ratios of these quotients from paired samples were calculated. We judged that a tumor had allelic imbalance when the calculated ratio was >1.67 or <0.6.

Statistics Statistical analyses of pathological classification numbers were made with the χ^2 test. Prognoses of patients were compared by means of Kaplan-Meier analysis with the log-rank test. The calculations were done with StatView Version 4.5 (Abacus Concepts, Inc., Berkeley, CA).

Table I. Frequency of Allelic Imbalances at Microsatellite Loci on 20p and 20q

Chr.	Arm	Loci	Informative	Allelic imbalance	(%)[a)]
20	p	*D20S103*	28	11	(39.3)
		D20S117	50	12	(24.0)
		D20S448	56	10	(17.9)
		D20S186	28	5	(17.9)
		D20S1145	41	9	(22.0)
		D20S112	50	9	(18.0)
	q	*D20S438*	50	11	(22.0)
		D20S55	59	14	(23.7)
		SRC11	47	7	(14.9)
		D20S119	53	10	(18.9)
		D20S100	58	8	(13.8)

a) Allelic imbalance/informative×100.

RESULTS

Table I lists the number of constitutional heterozygotes as well as tumors with allelic imbalance at loci on Chr. 20 among 70 cases of bladder cancers. Twenty-six out of 70 patients (37.1%) showed at least one allele imbalance at these loci. We found that 11 out of 28 informative samples (39.3%) had allelic imbalance at *D20S103*; however, at *D20S100*, only 8 out of 58 informative samples (13.8%) did. Table II shows the distribution of haplotypes. Eight patients showed imbalances only on the 20p arm and an additional 4, only on the 20q arm. Fourteen cases had allelic imbalances on both the short and long arms of Chr. 20. In particular, 8 cases were judged to have imbalances throughout the full length of Chr. 20. This map suggests 17 imbalances between *D20S1145* and *D20S112* on 20p and 16 imbalances between *D20S438* and *D20S55* on 20q. Both regions were close to the centromere of Chr. 20. Finally 7 microsatellite instabilities (MI) were detected. Patient 3 shown in Table II had MI at two loci. Patient 7 and 12 had both MI and imbalances. Three other MIs were detected separately in independent patients.

The relationships between allelic imbalances and several prognostic factors of the tumors were examined. Table III shows the numerical classification of the tumors according to the WHO grading criteria and TNM classification system. Allelic imbalances were found in 7 of 14 grade 3 (G3) tumors, but in only 17 of 53 G1 or G2 tumors. For pT factor, there was a tendency for higher-grade tumors to have more frequent genetic imbalances. However, this tendency was not significant.

We analyzed the survival period of the patients. The survival period was defined as the period in months from the operation yielding the tumor sample until the patient's death. The range of the observation period was 2 to 103 months, with an average of 33.8±29 months. In this

Table II. Deletion Map of Chromosome 20 in Human TCC

Locus	cM	Patients																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
D20S103	0.00	●	●	●	◎	◎	●	●	◎	●	●	●	●	◎	◎	●	●	◎	◎	◎	◎	◎	◎	◎	◎	◎	◎
D20S117	2.83	◇	◎	◎	◎	●	●	●	◎	●	●	◎	◆	●	●	●	●	●	◎	◇	◎	◇	◇	◇	●	●	◇
D20S448	18.79	◎	◇	◇	◎	●	●	●	●	◇	●	◎	◎	◎	●	●	◎	●	●	●	◇	◇	◇	◇	◎	◎	◇
D20S186	32.30	◎	◎	◆	◎	◎	◎	◆	◎	◎	●	●	◎	◎	●	●	●	◎	◎	◎	◎	◎	◎	◎	◎	◎	◇
D20S112	39.25	◇	◇	◎	●	●	●	◎	●	●	◎	●	◎	◎	●	◎	◎	●	◎	●	◇	◇	◇	◇	◇	◎	◎
D20S1145	46.70	◎	◇	◎	◎	●	◎	●	●	◎	●	◎	●	◎	◎	◎	◎	●	●	●	●	◇	◎	◎	◇	◎	◇
D20S438	54.09	◎	◇	◎	◇	◇	◎	◎	●	◎	●	●	●	●	◎	●	●	●	●	◎	◇	●	◎	◇	◇	◇	●
D20S55	58.00	◎	◇	◆	◇	◎	◎	●	●	◎	●	●	●	●	●	●	●	●	●	◇	◎	●	●	●	◎	◇	◇
SRC11[a)]	—	◇	◇	◎	◎	◇	◇	◇	◇	●	◎	●	●	●	◎	●	●	●	◎	◇	◇	◇	◎	◇	◎	◇	◇
D20S119	61.77	◇	◇	◇	◇	◇	◇	◇	◎	◇	◇	●	●	●	●	●	◎	●	●	◎	◇	◎	◇	◇	●	●	●
D20S100	84.78	◇	◎	◎	◇	◇	◇	◇	◇	◇	◇	◎	●	●	●	●	●	●	●	◇	◇	◎	◎	◎	◎	●	◇

a) 20q 11.2.
● allelic imbalance, ◎ no information, ◇ retention of two allele, ◆ microsatellite instability, ▭ region suspected to be deleted.

Table III. Correlation of Allelic Imbalance to Histological Classification of TCC

		No. of patients	No. of allelic imbalance (%)		
			20p	20q	Overall
All		70	22 (31.4)	18 (25.7)	26 (37.1)
Grade	G1	18	4 (22.2)	5 (27.8)	6 (33.3)
	G2	32	9 (28.1)	6 (18.8)	10 (31.3)
	G3	14	5 (35.7)	4 (28.6)	7 (50.0)
pT	A	30	8 (26.7)	8 (26.7)	11 (36.7)
	1	17	4 (23.5)	2 (11.8)	4 (23.5)
	2	3	0 (0.0)	1 (33.3)	1 (33.3)
	3	9	3 (33.3)	3 (33.3)	4 (44.4)
	4	3	1 (33.3)	0 (0.0)	1 (33.3)

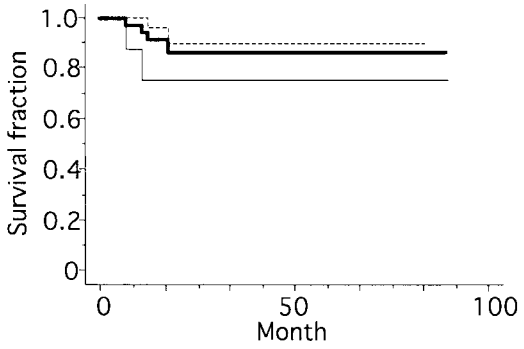

Fig. 1. Survival rate of patients with TCC resected by TUR operation. A Kaplan-Meier survival plot of the patients who have TCCs taken under TUR-Bt operation is shown (n=37: bold line). The patients are divided into two groups, i.e., tumor with (n=8: fine line) or without (n=29: broken line) allelic imbalance of the long arm of chromosome 20.

period, 13 patients died of cancers and 8 died of other causes. The presence of allelic imbalances on either or both the 20p and 20q arms, however, was not correlated with differences in the length of survival as determined by the Kaplan-Meier method (data not shown). Fig. 1 shows a Kaplan-Meier survival plot for the patients who had a TUR operation (n=37). This graph compares the survival curves of the patients bearing tumors with or without allelic imbalance on loci of 20q. The survival of patients with allelic imbalances on 20q tended to be shorter, but this tendency was not significant (P=0.16). Much less significance was observed when the survival curves were compared between patients with or without allelic imbalances on the whole 20 or 20p (data not shown). On the other hand, the tumor grade showed a significant effect on the survival period in a log-rank test (P=0.057).

DISCUSSION

The present study revealed a moderate incidence of allelic imbalances on human Chr. 20 of patients with TCC, one of the most common tumors in humans. The survey of this chromosomal region was carried out because of the observation of quantitative trait loci affecting the cancer survival period in the homologous chromosomal region of a mouse model. The longer survival period is determined either by higher host resistance and/or by lower prolifera-

tive properties of the tumor. Loss of suppressor genes may cause tumor progression. In this study, we used fluorescein-labeled PCR products to detect allelic imbalances of cancer cells. Although there may have been some contamination of normal tissues in the tumor specimens, the presence of allelic imbalances including gain and LOH was detected in over 37% of 70 TCC patients. Despite extensive studies of LOH in human bladder cancers, so far relatively little attention has been paid to Chr. 20. Gain of Chr. 20 is reported to be correlated with urothelial cell immortalization,[16] and screening with the comparative genomic hybridization method revealed multiple chromosomal imbalances including gain of Chr. 20.[17] Loss of tumor suppressor genes or abnormal gain of oncogenes may play a role in carcinogenesis. The oncogene *src* is mapped on the long arm of Chr. 20.[18] In this study, however, we did not see a remarkably high rate of imbalances at the microsatellite locus *src11*. Therefore, *src11* is not likely to be involved in the carcinogenic allelic imbalance on Chr. 20.

Chemically induced mouse bladder tumors are highly invasive and rapidly fatal to genetically susceptible hosts. However, in human urinary tract TCCs, the relationship between the presence or absence of genetic imbalance and clinico-pathological features was inconclusive. Our results using TUR-limited specimens left some possibility that there is a relationship between tumor progression and chromosomal changes. To determine whether the allelic imbalance on Chr. 20 is a prognostic factor for bladder cancers, further study with a larger number of categorized patients is required. In this study, samples were not limited to primary tumors, and over 80% patients had recurrence before or after sampling of DNA. It will be necessary to check patients' survival time from the first tumors and any genetic changes on Chr. 20, as they might have occurred in earlier stages of carcinogenesis. Genetic changes in TCC are maintained in recurrent tumors.[19] Newly added LOH may alter malignant cell property and induce tumor progression. In human TCC of the bladder, LOH at loci on Chr. 9 are the most frequent genetic alterations. In part of these 70 TCCs, our previous experiments had revealed genetic alterations on 9p (5/11) and 9q (23/45). However, synergistic effects of genetic alterations in these loci remain unclear.

The results of this study do not indicate that the genes involved in bladder cancers in both species are identical. However, it is still possible that some steps of target cell differentiation and transformation vulnerable to carcinogenic agents, or some steps of tumor progression, are shared by the two species. Using a similar approach, we were able to locate the chromosomal segments with frequent gene alterations in lung cancers. By examining the syntenic regions of responsible loci for mouse pulmonary adenoma, LOHs were found at high frequency in human lung cancers.[20, 21] Examination of various experimental models has revealed that there are considerable differences of the responses to carcinogenic agents among different strains of laboratory animals. Some of these differences, if not all, may represent polymorphism in the genes involved in certain critical steps of carcinogenesis. Although careful interpretation of results is imperative, genetic analysis of animal models should help us to find steps extrapolable to human cancers.

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