Chromosomal Localization of a Strain-Specific *p53*-Related Sequence in Rat (*Rattus norvegicus*)

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Abstract: Strain-specific restriction fragments hybridizing to human *p53* gene cDNA, which was detected in only particular strains of inbred rat were revealed by Southern blot hybridization of DNAs of various inbred rat strains. Chromosomal location of the strain-specific fragments was determined on rat chromosome 1 between *Kal* and *Pkc* loci by linkage analysis using microsatellite marker loci. The fragments were concluded to be a novel *p53*-related sequence which is present in particular rat strains and absent from the remaining strains.

Key words: linkage map, *p53*, rat

Introduction

Mutation of the *p53* gene is one of the most common genetic alterations found in a variety of mammalian tumors [10, 18, 20, 21]. The *p53* protein is initially reported to be a cellular tumor antigen which is commonly found in a variety of tumors and transformed cells [5, 22]. Recently, wild-type *p53* has been shown to reduce the tumorigenicity of transformed cells and suppress the transformation of cells by transforming genes [4, 8]. The *p53* gene, therefore, is believed to be a tumor-suppressor gene, of which inactivation is involved in tumorigenesis.

In addition to point mutations, various genomic alterations including deletions, loss of allelic heterozygosity, and rearrangements of the *p53* gene

have been detected in human, mouse and rat tumors [2, 6, 17]. In particular, a variety of mutations in the *p53* gene including the genomic rearrangements have been reported in chemically induced rat tumors [17, 18, 21], but there are several *p53*-related sequences that are present in rat genome [3, 24] which make it difficult to identify the genomic alteration of the *p53* gene. Therefore, findings directed to genomic structure of the rat *p53* gene and its related sequences as well as their chromosomal localization should be informative for an analysis of the genomic alteration in the *p53* gene. We have investigated localization of the *p53* gene and its related sequences in the rat chromosomes, and report here a novel *p53*-related sequence which is only present in particular strains of inbred rats, as well as its localization on rat chromosomes.

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Materials and Methods

Animals: DNAs extracted from 20 inbred rat strains described by Kunieda *et al.* [12] were used in the present study. For linkage tests, 57 backcross progeny were obtained from crosses of (WKS/Iar $\times$ IS/Iar) $\times$ WKS/Iar. High molecular weight genomic DNAs were prepared from the livers of the rats by phenol extraction.

Southern blot hybridization: Plasmid pSP53 containing a 2.0-kb fragment of human *p53* gene cDNA [19] was digested with *Eco*RI and *Bam*HI. The 2.0-kb inserted fragment was purified by agarose gel electrophoresis, labeled by random priming [7] to a specific activity of 2×10^6 cpm/ng DNA with [α - 32 P] dCTP, and used as a probe. The genomic DNAs (10 μ g) were digested at 37°C for 8 hr with 100 units of *Eco*RI, *Bam*HI, *Hind*III, *Pst*I, or *Pvu*II endonucleases, and separated by 0.7% agarose gel electrophoresis. After denaturation and neutralization, the DNAs were transferred to a Nylon-membrane filter. The filters were hybridized with hybridization solutions (50% formamide, $5 \times$ SSC, 1 mM EDTA, 0.5% SDS, $5 \times$ Denhardt's solution, and 100 μ g/ml of sonicated herring sperm DNA) containing the 32 P labeled probe at 50°C for 24 hr and washed three times in $1 \times$ SSC, 0.1% SDS at room temperature for 30 min each time and once in $0.1 \times$ SSC, 0.1% SDS at 50°C for 1 hr. The filters were then exposed to Kodak XAR-5 film.

Typing of marker loci: Segregation of six biochemical marker loci (*Mup-1*, *Svp-1*, *Acp-2*, *Cs-1*, *Es-6*, and *Es-8*), three RFLP loci (*Gh*, *Nras-1*, and *Nras-2*) and three coat-color loci (*A*, *H*, and *C*) in the backcross progeny were determined as previously reported [12]. Seventeen microsatellite loci [13, 23] were analyzed by PCR amplification. The PCR was performed in 50 μ l of a reaction mixture containing 0.1 μ g of the genomic DNA, 0.4 μ g each of the oligonucleotide primers, 200 μ M each of dGTP, dATP, dTTP, and dCTP, 2.5 u of *Taq* polymerase (Biotech), and the reaction buffer recommended by the manufacturer. Forty cycles of amplification each consisting of denaturation for 60 sec at 94°C, annealing for 150 sec at 60°C, and extension for 150 sec at 72°C were performed in a Thermal Sequencer TSR-300 (Iwaki glass). The products of the PCR were electrophoresed on 8% polyacrylamide gel in TBE buffer and visualized with ethidium bromide.

Results

In a series of Southern blot hybridization with different restriction endonucleases, the rat DNA gave five to eight fragments hybridizing to the human *p53* cDNA probe, whereas human and mouse DNAs gave only two or three fragments in either of the enzyme digests. As shown in Fig. 1, four fragments, 10, 9, 4.4 and 1.8 kb, in the *Eco*RI digests and six fragments, >23, 20, 14, 10, 6, and 4 kb, in the *Bam*HI digests were observed in all strains. In addition to these common fragments, the SDJ/Hok and TO/Hok strains (lanes 3 and 5, respectively) gave fragments of 3.0 and 1.5 kb in the *Eco*RI digests, and 4 and 2.3 kb in the *Bam*HI digests, which were absent from the other three strains. It is likely that the 4-kb band in the *Bam*HI digest of the SDJ/Hok and TO/Hok strains, which are more intense than that of other strains, composed two fragments. Additional fragments of 9 and 4 kb, 6 and 5kb, and 3 kb were also observed in the *Hind*II, *Pst*I and *Pvu*II digests of the SDJ/Hok and TO/Hok strains, respectively (data not shown). The additional fragments of the each enzyme digest gave more intense bands than the other common fragments. The presence and absence of the additional fragments in the 25 inbred strains are shown in Table 1.

In the backcross of (WKS/Iar $\times$ IS/Iar) F_1 $\times$ WKS/Iar, all F_1 progeny gave the additional fragments while IS/Iar gave the additional fragments and WKS/Iar gave no additional fragments, and the 57 backcross progeny segregated into 27 rats having the additional fragments and 30 rats having no additional fragments. The ratio of the numbers of the rats in each type did not significantly differ from the 1:1 ratio and there is no significant difference between the ratio of male to female rats in each type. These findings indicate an autosomal dominant trait of the transmission of the additional fragments. We designated the locus of the strain-specific fragments *Trp53l4*.

The possibility of linkage between the *Trp53l4* locus and 26 marker loci on rat chromosomes 1, 2, 3, 4, 5, 7, 8, 9, 10, 13, and 19 was determined in the same backcross progeny. As shown in Fig. 2, significant linkages were observed between the *Trp53l4* locus and the loci on rat chromosome 1 including *Pkc* (Protein kinase C type I), *Kal* (Kallikerin, renal), *c* (albinism), and *Nras2*

Fig. 1. Autoradiograms of *Bam*HI (A) and *Eco*RI (B) fragments in DNAs from five inbred rat strains hybridized with the human *p53* cDNA probe. DNAs from inbred rats were digested with *Eco*RI or *Bam*HI and analyzed as described in the text. 1. LEA/Hok, 2. LEC/Hok, 3. SDJ/Hok, 4. LEJ/Hok, 5. TO/Hok, 6. mouse, 7. human. Lengths of the marker fragments are shown on the right.

Table 1. Distribution of the strain-specific fragments of *p53*-related sequence in inbred rat strains

Strains	
Present	IS/Iar, SDJ/Hok, TO/Hok, TOM/Iar
Absent	ACI/Hok, ALB/Hok, BEG/Iar, BN/Hok, BUF/Hok, COP/Iar, F1R/Iar, F344/Hok, KYN/Hok, LEA/Hok, LEC/Hok, LEJ/Hok, LEO/Iar, LET/Iar, NIGIII/Hok, NIGIII/Iar, TU/Iar, TX/Iar, W/Hok, WKS/Iar, WM/Iar

loci [12–14, 23]. The recombination frequency between *Trp53l4* and *Pkc*, *Trp53l4* and *Kal*, and *Pkc* and *Kal* was $5.3 \pm 3.0\%$, $11 \pm 4.0\%$, and $16 \pm 4.9\%$, respectively. We determined the location of the *Trp53l4* locus between the *Kal* and *Pkc* loci of rat chromosome 1 by

minimizing the number of recombination events. The linkage map of the rat chromosome 1 obtained in the present study is shown in Fig. 3.

Discussion

The number of copies of *p53* gene and its related sequences per haploid genome is known to differ in several mammalian species. At least three *p53*-related sequences have been reported in rats [3, 15, 25], while a functional *p53* gene alone, and one functional gene and one processed pseudogene have been reported in human and mouse, respectively [16, 26]. The rat *p53* gene itself is located on chromosome 10, and three related sequences have been reported to localize on chromosomes 9, 14, and 18, respectively [25]. There-

Fig. 2. Segregation of five loci in backcross progeny. Each column represents the types of chromosomes identified in the backcross progeny that were inherited from the (IS/Iar $\times$ WKS/Iar) F_1 parents. Black boxes represent the IS/Iar type and open boxes represent the WKS/Iar type. The number of backcross progeny carrying each type of chromosome is listed at the bottom.

fore, the strain-specific fragments, which are located on the chromosome 1 were concluded to be a novel *p53*-related sequence different from the *p53*-related sequences which have been already reported in the rat.

Generation of novel sequences homologous to particular genes in eukaryotic genome is expected to be caused by several mechanisms. Such mechanisms include an unequal crossing-over in tandemly repeated sequences and an integration of a cDNA of a particular mRNA to form a processed pseudogene. Polymorphisms in the presence or absence of the particular sequences caused by the former have been reported in the human globin gene family [9, 11] and those caused by the latter have been reported in the human dihydrofolate reductase (DHFR) gene [1]. Because the chromosomal location of the strain-specific *p53*-related sequence is different from those of the *p53* gene and other *p53*-related sequences and the strain-specific sequence hybridizes to the *p53* cDNA probe more intensively than the other sequences, the latter rather than the former is likely to be responsible for the strain-specific sequence.

Recently, variation of a *p53*-related sequence involving absence of the *p53* pseudogene in particular inbred strains of mouse (*Mus musculus*) has been revealed, and a similar variation has also been observed in wild mouse, *Mus spretus* (Kominami, personal communication). Although the chromosomal location of the pseudogene of the mouse does not correspond to those of the *p53*-related sequences of the rat, it is of specific interest that there are similar variations with regard to the presence or the absence of the particular *p53*-re-

Fig. 3. Linkage map of rat chromosome 1 near the *Trp53l4* locus based on the recombination frequency determined in the present study.

lated sequences in *Rattus norvegicus*, *Mus musculus*, and *Mus spretus*.

Mutations of the *p53* gene have been reported for various chemically induced rat tumors [18, 21]. In addition to point mutations, large scale DNA rearrangements including the appearance of novel fragments which can be detected by Southern blot hybridization have also been reported [6, 17]. The present finding of the strain-specific *p53*-related sequence should be valuable in correctly evaluating possible rearrangements of the *p53* gene in rat tumors.

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