Submitted to Genomics - Brief Report

OCT 19 1993

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**Identification of a Polymorphic Site as a Mutational Site
in Exon VI of the Mouse p53 Gene**

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July 1993

Subject Category: Disease Mutations

Running Title: Mutational Site in p53 Exon VI

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Sequencing by hybridization techniques (1) are being used to analyze the incidence of specific p53 mutations associated with radiation-induced and spontaneous lymphosarcomas in mice. One sequence difference noted as being a mouse strain-specific polymorphism has been identified through these experiments as being a mutational, rather than a polymorphic, site.

Southern blots of various p53 exon DNAs produced by PCR were hybridized to [$\gamma^{32}\text{P}$]ATP-labeled oligonucleotides. Table I contains the PCR amplification sequences, oligonucleotide probes, and other details. The reaction conditions (see ref. 1; 10 ng probe per 1 mL hybridization buffer [0.5 M Na_2HPO_4 , 7% wt/vol sodium lauroyl sarcosine] at 12 °C for 16 h, followed by a 40-min wash at 12 °C in 4x standard saline citrate) were designed to enable oligomer probes to hybridize with full-match target sequences several-fold more efficiently than with mismatched targets. We designed a set of oligonucleotide probes which could be used to detect variant sequences in mutational hotspots that have been shown to be associated with p53 oncogenic changes in different tumor systems (2). As a model for optimizing the method, we used p53 exon VI sequences of C57BL/6 and BALB/c mouse strains. Bienne *et al.* (3) had reported a strain-specific nucleotide polymorphism that occurs as a Met codon at amino acid position 234 in the p53 protein of the BALB/c mouse and as an Ile codon in p53 from the C57BL/6 mouse strain. This was described as one of several mouse strain-specific polymorphisms in the p53 gene.

According to these reported polymorphisms (3), probes S1 and A1 (see Table 1) would hybridize only to BALB/c exon VI p53 sequence, whereas probes S2 and A2 would have a full-match target in the C57BL/6 exon VI p53 DNA and in one or two additional *RAG-1* control DNA sequences (see sequences in Table 1). As shown in Fig. 1, however, both BALB/c and C57BL/6 mice have a sequence coding for a Met codon for amino acid position 234 of the p53 protein. It should be noted that the oligomers hybridized appropriately to control (*RAG-1*) sequences, based on the sequences reported by Oettinger *et al.* (4).

Our findings agree with the proposed p53 consensus amino acid sequence. Since amino acid position 234 is a methionine residue in human, monkey, rat, chicken, *Xenopus* (p53 genes A and B both), and rainbow trout (2), it would be surprising if there was a mouse strain-specific difference at this site. It is interesting that the original sequence reported for exon VI of mouse p53 was derived from the SVT2 cell line, an SV40-transformed fibroblast line. It is likely that this Met→Ile change is associated with the transformation of the cell line and that the change may be oncogenic for p53. Furthermore, other amino acid alterations of this codon of p53 have been reported in three out of 280 cases of different human tumors (2) and in one of 11 mouse leukemia T cell lines (5). Our findings suggest that the reported Met/Ile polymorphism reported in codon 234 of mouse p53 is actually an oncogenic change associated with the transformed SVT2 cell line.

ACKNOWLEDGEMENTS

Work supported by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract No. W-31-109-ENG-38.

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Table 1. Experimental Details

Lane in Fig. 1	Template source genomic data- (mouse strain)	Primer pairs	PCR products	Target sequences contained in PCR products (PRESUMED)	Should hybridize to probe mixes	Relative amounts of hybridization with probes*				Target sequences as confirmed by hybridization (ACTUAL)
						S1	A1	S2	A2	
1	BALB/c	CTTCCCCAGGCCGGCTCTGA GAGTCTTCCAGTGTGATGAT	exon VI of p53 gene 669-773 bp	5'TACATGTG ATGTACAC5'	S1:5'NNTACATGTN3' A1:5'NNCACATGTA3'	11.5	3.6	1	1.5	Presumed sequences confirmed
2	C57BL/6	CTTCCCCAGGCCGGCTCTGA GAGTCTTCCAGTGTGATGAT	exon VI of p53 gene 669-773 bp	5'TACATCTGT ATGTAGACA5'	S2:5'NNTACATTN3' A C A2:5'NACATATGTA3' A G	8.3	3	1.25	1.5	TACATGTG Actual sequences defined
3	BALB/c	CTATTGTCCCTCTTGCCGAT GGTTGAAAAATCTGCCTCCC	fragment of RAG-1 gene 1060-1571 bp	5'TACATCT ATGTAGA5'	S2:5'NNTACATTN3' A C	1	1	10	1	Presumed sequences confirmed
4	BALB/c	TA1TGTCCCTCTTGCCGAT GCCTGCCATCTCTTCCTTC	fragment of RAG-1 gene 1060-2586 bp	5'TACATCT ATGTAGA5' 5'TACATCTGT ATGTAGACA5'	S2:5'NNTACATTN3' A C A2:5'NACATATGTA3' A G	1.7	1.3	9.9	35.5	Presumed sequences confirmed

*The data were quantified by ImageQuant software, and relative values were obtained by dividing so as to assign a value of 1 to the least intensive hybridization signal.

FIGURE LEGEND

Fig. 1: For all sections of this figure (a-d), lanes contain PCR-amplified DNA for (1) BALB/c p53 exon VI, (2) C57BL/6 p53 exon VI, (3) BALB/c *RAG-1* fragment 1060-1571, (4) and BALB/c *RAG-1* fragment 1060-2586 (see Table 1). Blots were hybridized to oligonucleotide mixes and then were exposed against storage phosphor plates for 24-72½ h and scanned in a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Block a, S1 (NNTACATGTN); b, A1 (NNCACATGTA); c, S2 (NNTACATHTN); d, A2 (NACADATGTA). Abbreviations: N = equimolar amounts of A,T,G,C; H = equimolar amounts of A,T,C; D = equimolar amounts of A,T,G. Numbers: 1,2 = position of bands in lanes 1 and 2; 3 = position of band in lane 3; 4 = position of band in lane 4 on all four blots.

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