Mutation Spectrum of the p53 Gene in Bone and Soft Tissue Sarcomas

Junya Toguchida, Yoshikazu Yamaguchi, Bruce Ritchie, Roberta L. Beauchamp, Siri H. Dayton, Guillermo E. Herrera, Takao Yamamuro, Yoshihiko Kotoura, Masao S. Sasaki, John B. Little, Ralph R. Weisschnei, Kanji Ishizuki, and David W. Yandell

Howe Laboratory of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, Massachusetts 02114; J. B. L., D. W. Y.; and Department of Orthopaedic Surgery (J. T., To., Y., Ta., Y., K.) and Radiation Biology Center (To. Y., M. S., S., K. I.), Kyoto University, Kyoto 606, Japan; Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7 (B. R.); Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115; J. B. L., D. W. Y.; and Department of Radiation and Cellular Oncology, University of Chicago Medical Center, Chicago, Illinois 60637 (R. R. W.)

ABSTRACT

We present here an analysis of the spectrum of mutations of the p53 gene seen in 127 bone and soft tissue sarcomas of various histological classifications. Gross rearrangements were analyzed by Southern blotting using a complementary DNA probe from the p53 gene, and subtle alterations in the entire coding sequence (exons 2 through 11) were identified by a combination of single-strand conformation polymorphism analysis and direct genomic sequencing. A total of 42 somatic alterations of the p53 gene were found, of which 21 were gross rearrangements and 21 were subtle alterations. These included 17 cases of a single base substitution, 3 small deletions, and one single base insertion. In contrast to reported findings for other types of cancer, we found that mutations of the p53 gene in sarcomas are quite heterogeneous both in their distribution throughout the gene and in the type of genetic alterations that result. All 13 missense mutations we found occurred at highly conserved residues, whereas 8 nonsense mutations occurred at sites that spanned the gene from codons 46 to 316. Surprisingly, approximately one-half of the osteosarcomas with allelic deletions on 17p did not have detectable alterations in the coding sequence of the p53 gene.

INTRODUCTION

The p53 gene, located at chromosome 17p13, encodes a Mr 53,000 nuclear phosphoprotein that has been characterized extensively. Several studies have shown that reintroduction of a wild-type p53 gene into p53-deficient cells leads to suppression of the neoplastic phenotype; hence this gene has been categorized as a tumor suppressor (1, 2). This suppressor activity may be exerted through the transcriptional control of proliferation-related genes, and several experiments have identified a transcription-activating sequence in the p53 protein (3, 4). Another possible function of this protein is the regulation of DNA replication through its association with DNA replication complexes. In simian virus 40-transformed cells, the p53 protein has been shown to bind large T-antigen, inhibiting T-antigen complexes. In simian virus 40-transformed cells, the p53 protein

Received 2/27/92; accepted 9/11/92.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by grants from the NIH (D. W. Y., R. R. W.), the Center for Radiation Therapy (D. W. Y., R. R. W.), and the Japanese Ministry of Education, Science and Culture (M. S. S., Y.). D. W. Y. is a Research to Prevent Blindness Dolly Green Scholar.

2 To whom requests for reprints should be addressed, at Howe Laboratory, Molecular Genetics, Room 575, Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA 02114.

These include a high mutation frequency at CpG sites in colorectal cancer (10) and lymphoid malignancies (11), a predominance of G to T transversions in non-small cell lung cancer (12), and a predominance of G to T transversions at codon 249 in hepatocellular carcinomas (13, 14). Osteosarcoma, a representative of malignant tumors derived from bone and soft tissue, is one of the first human tumors in which p53 gene mutations were found (15). We describe here a comprehensive study of the spectrum of p53 gene mutations found in a large number of bone and soft tissue sarcomas. Our goal was to compare the spectrum of p53 mutations in sarcomas with that seen in other tumors in the hope that useful information about the role of p53 in sarcoma progression could be found.

MATERIALS AND METHODS

DNA Samples. Tumor tissues were obtained at surgical resection, dissected, rinsed to remove adjacent normal tissue and blood, flash frozen, and stored at −70°C until DNA extraction. Nonmalignant infiltrate or stromal cell content was not quantitated, but histopathological examination was carried out, and only those samples that contained predominantly neoplastic cells were used for molecular studies. High-molecular-weight DNA from tumor tissues and peripheral leukocytes were prepared as described elsewhere according to standard protocols (16).

Southern Blot Analysis. Gross rearrangements of the p53 gene were analyzed using a cDNA3 probe for the p53 gene (pR4-2) (17). This cDNA probe hybridizes with three EcoRI-digested fragments (15, 15, and 3.5 kilobases) (18). The 3.5-kilobase fragment, corresponding to exon 1 sequences, is too faint to be seen on normal autoradiographic exposure. The presence of these fragments was confirmed by additional, longer exposure of all blots (data not shown). Allelic deletions on 17p were analyzed using six polymorphic probes (p10.5, pA10-41, pH92-1, pmCT35-1, pmCT35-2, and pYN222) as previously described (19, 20). Restriction endonuclease digestion of DNA samples, agarose gel electrophoresis, Southern hybridization, labeling of probes, and autoradiography were performed by standard procedures (16).

SSCP Analysis. Each primer sequence was obtained from published data or by sequencing genomic clones of the human p53 gene provided by Dr. L. Crawford. PCR fragments were generated from 50 ng of genomic DNA in a 50-μl mixture containing: 20 μM dATP, dTTP, dGTP; 2 μM dCTP; 1.0 to 2.5 mM MgCl2; 20 pmol of each primer; 20 mm Tris (pH 8.4 or 8.6); 50 mM KCl; 50 μg/ml bovine serum albumin; 0.5 units Taq polymerase (Perkin-Elmer Cetus); and 0.1 μl (7 nm) [α-32P]dCTP (3000 Ci/mm). PCR was carried out using 30 cycles (30 s at 94°C, 30 s at 52° to 60°C, and 120 s at 71°C) on a programmable thermal controller (MJ Research, Inc.). One-tenth of the amplified product was diluted with 15 to 40 μl of a 0.1% sodium dodecyl sulfate-10 mm EDTA mixture, followed by 1:1 dilution with 5% formamide, 89 mm Tris, 2 mm EDTA, 89 mm boric acid, 0.05% bromophenol blue, 0.05% xylene cyanol loading solution. Dilute samples were heat denatured at 95°C for 2 min and then were loaded on 6% nondenaturing polyacrylamide gels: once on a gel containing 10% glycerol, and a second time on a gel that did not contain glycerol.

The abbreviations used are: cDNA, complementary DNA; SSCP, single-strand conformation polymorphism; PCR, polymerase chain reaction.
Electrophoresis was performed at room temperature with a constant power of 8 W for 8 to 16 h. Autoradiography was carried out for 12–24 h without an intensifying screen.

Direct Genomic Sequencing. Direct sequencing of double-stranded PCR fragments was performed as previously described (21). Amplified PCR products were purified through Sepharose CL-6B (Pharmacia) and combined with 7 pmol of 32P-end-labeled primer. Primer-template mixtures were heat-denatured, and sequencing reactions were carried out with Sequenase (U.S. Biochemical). Electrophoresis was performed on 6% denaturing polyacrylamide gels. Autoradiography was carried out for 12–16 h without the use of an intensifying screen.

RESULTS

Rearrangements of the p53 Gene. DNA from 127 bone and soft tissue sarcomas was examined by Southern blot analysis using a cDNA probe from the p53 gene (pR4-2) (17). The pathological diagnosis for these samples was: osteosarcoma in 76 cases; malignant fibrous histiocytoma in 13 cases; chondrosarcoma in 9 cases; synovial sarcoma and Ewing’s sarcoma (5 cases each); liposarcoma and rhabdomyosarcoma (4 cases each); chordoma in 3 cases; fibrosarcoma in 2 cases; malignant schwannoma; alveolar soft part sarcoma; leiomyosarcoma; malignant hemangiopericytoma; epithelioid sarcoma; and teratoma (one case each). Gross rearrangements of the p53 locus were detected in 21 tumors (Table 1). These gross rearrangements were identified by an abnormal restriction pattern, usually involving the presence of additional fragments of abnormal size (Fig. 1). Abnormally migrating bands in Fig. 1 have arisen from structural rearrangements within a 15-kilobase EcoRI fragment containing exons 2–10 of the p53 gene; the remaining faint 15-kilobase band is due to a second EcoRI fragment containing the unrearranged 3’ end of the gene (18). In all cases the rearrangements were not observed in DNA of leukocytes taken from the same patient, excluding the possibility of neutral polymorphisms at the enzyme recognition sites (data not shown). Of the 21 rearrangements we found, 18 occurred in osteosarcoma tumors, 2 in malignant fibrous histiocytomas, and 1 in a fibrosarcoma.

Screening for Point Mutations of the p53 Gene. Mutations too small to be resolved by Southern blotting were identified in two steps. In the first step, SSCP analysis was used to identify samples with DNA sequence variations. SSCP was performed with only minor modifications of the original published procedure (22). Separate pairs of oligonucleotide primers were used to amplify each of the p53 exons 2–11 except exon 5, for which two overlapping PCR fragments were analyzed (Table 2). The exon-containing PCR fragments included at least 10 base pairs of 5’ and 3’ flanking sequences surrounding each exon. To increase the sensitivity of the SSCP technique for some regions, PCR-amplified DNA fragments from some exons were digested by an appropriate restriction endonuclease to reduce the size of the PCR fragments to approximately 200 base pairs (Table 2). To further characterize variants, samples that showed a variant allele by SSCP analysis were analyzed by direct genomic sequencing (21). To confirm that the majority of mutations in the p53 gene were detected by this screening process, we carried out DNA sequence analysis on 36 “SSCP-negative” samples in exon 5 and 48 samples in exon 7. This analysis revealed no additional point mutations (data not shown).

A total of 21 subtle alterations including point mutations, small deletions, and an insertion were identified and characterized by this screening method (Table 3). Examples of SSCP analysis and sequences showing mutations are presented in Figs. 2 and 3, respectively. Of these 21 mutations, 14 were found in osteosarcomas, 4 occurred in chondrosarcomas, 2 in malignant fibrous histiocytoma, and one mutation was found in a liposarcoma. None of these tumors had a gross alteration of the p53 gene except one case (KS-103), in which a heterozygous rearrangement was found. The point mutation found in this tumor was also heterozygous. These results suggest that two different mutations occurred in each of two copies of the p53 gene in this case. Although we cannot exclude the possibility that two mutations occurred on the same allele, we believe that this is unlikely.

Characteristics of Point Mutations Found in Sarcomas. The locations of the 21 point mutations we found are shown in Fig. 4. Of these, 13 mutations were single-base changes that cause amino acid substitutions in the p53 protein, and 6 are nonsense mutations that result in premature termination codons due to deletion, insertion, or the direct creation of a stop codon due to a single base substitution. The remaining two mutations were intronic mutations at canonical splice junctions. The majority of p53 gene mutations in various types of cancers were found in five evolutionarily conserved domains (8, 9, 23). Of the 21 point mutations described in this study, 10 (48%) occurred in the conserved domains; all 10 of these were missense mutations.

Three more missense mutations (162Leu to 162Pro, 193His to 193Gln, 259Asp to 259Val) were found outside the conserved domains but occurred at codons that are identical in human,
Table 2 Oligonucleotide primer pairs to amplify SSCP fragments of each exon in the p53 gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Primer sequence (sense/antisense)</th>
<th>Size of amplified fragment (base pairs)</th>
<th>Restriction endonuclease</th>
<th>Size of digested fragment (base pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5'-TGGAAAGTCTCATTAGCCTGGA 5'-CGAGATTGTTCTTCCGAGAA</td>
<td>281</td>
<td>BamHI</td>
<td>132,131,18</td>
</tr>
<tr>
<td>3</td>
<td>5'-AGCCAAATTCCTAGGGGACT 5'-TTGGAATTTGCCTGCC 5'-CTCATGTGTCGTGGTCTCTGG</td>
<td>184</td>
<td>No digestion</td>
<td>184</td>
</tr>
<tr>
<td>4</td>
<td>5'-ACTTCCTGAAACACCGTCT 5'-CAGCATTGAACTCCTGATGG</td>
<td>440</td>
<td>MspI</td>
<td>176,159,105</td>
</tr>
<tr>
<td>5 (5' end)</td>
<td>5'-TTATCTGTTCCTAGTGGAAC 5'-CTCATGTGGCTGACTCTTGG</td>
<td>189</td>
<td>No digestion</td>
<td>189</td>
</tr>
<tr>
<td>5 (3' end)</td>
<td>5'-TTTCCACACCCCCCGGGCAGCA 5'-ACCGGGGGAGGACCACGGCCCAGGG 5'-CTCCACAGACCCAGTTGAC</td>
<td>162</td>
<td>No digestion</td>
<td>162</td>
</tr>
<tr>
<td>6</td>
<td>5'-ACGCAAGCGGCTGTTGCCCA 5'-CTCCACAGACCCAGTTGAC 5'-TCCATTGCTTGGGACGGCAC</td>
<td>201</td>
<td>No digestion</td>
<td>201</td>
</tr>
<tr>
<td>7</td>
<td>5'-GGGTCCTCATTGCGCCCTGTTG 5'-CAGTGTGCAGGGTGGCAAGT 5'-CTGCCTCTTGCTTCTCTTTT</td>
<td>171</td>
<td>No digestion</td>
<td>171</td>
</tr>
<tr>
<td>8</td>
<td>5'-CTGCCTCTGCCCTGCTCTCCTTTGT 5'-TTCCTCACCAGGCTCTTGT</td>
<td>204</td>
<td>No digestion</td>
<td>204</td>
</tr>
<tr>
<td>9</td>
<td>5'-GCAAGTATGCGCTAGATCC 5'-GCAGTTATGCCTCAGATTCA</td>
<td>185</td>
<td>No digestion</td>
<td>185</td>
</tr>
<tr>
<td>10</td>
<td>5'-GTTACTGTGTATATATATTAC 5'-ATGAGAATGGAATCCTATGG 5'-AGACCCTCTCATCAGTGA</td>
<td>117</td>
<td>No digestion</td>
<td>117</td>
</tr>
<tr>
<td>11</td>
<td>5'-GGGAGGGGGAGGCTGTCAGTG 5'-AGGAGAGGAGGCTGTCAGTG</td>
<td>178</td>
<td>No digestion</td>
<td>178</td>
</tr>
</tbody>
</table>

Table 3 Somatic point mutations of the p53 gene in sarcomas

<table>
<thead>
<tr>
<th>ID</th>
<th>Tumor type</th>
<th>Exon</th>
<th>Codon</th>
<th>Within conserved domains?</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Wild-type allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS-146</td>
<td>OS</td>
<td>4</td>
<td>46–7</td>
<td>No</td>
<td>1 base pair deletion</td>
<td>Stop at 122</td>
<td>-</td>
</tr>
<tr>
<td>KS-131</td>
<td>OS</td>
<td>4</td>
<td>112</td>
<td>No</td>
<td>1 base pair deletion</td>
<td>Stop at 122</td>
<td>-</td>
</tr>
<tr>
<td>KS-57</td>
<td>OS</td>
<td>5</td>
<td>162</td>
<td>No</td>
<td>ATC to TTC</td>
<td>Ile to Phe</td>
<td>+</td>
</tr>
<tr>
<td>KS-93</td>
<td>OS</td>
<td>5</td>
<td>173</td>
<td>Yes</td>
<td>GTG to GCG</td>
<td>Val to Ala</td>
<td>-</td>
</tr>
<tr>
<td>308-OT</td>
<td>OS</td>
<td>5</td>
<td>175</td>
<td>Yes</td>
<td>GCC to CAC</td>
<td>Arg to His</td>
<td>+</td>
</tr>
<tr>
<td>KS-134</td>
<td>OS</td>
<td>6</td>
<td>193</td>
<td>No</td>
<td>CAT to CAA</td>
<td>His to Gin</td>
<td>-</td>
</tr>
<tr>
<td>KS-133</td>
<td>MFH</td>
<td>6</td>
<td>196</td>
<td>No</td>
<td>CCA to TGA</td>
<td>Arg to Stop</td>
<td>+</td>
</tr>
<tr>
<td>KS-211</td>
<td>OS</td>
<td>6</td>
<td>221</td>
<td>No</td>
<td>GAG to TAG</td>
<td>Glu to Stop</td>
<td>+</td>
</tr>
<tr>
<td>KS-140</td>
<td>OS</td>
<td>7</td>
<td>227–8</td>
<td>No</td>
<td>4 base pair deletion</td>
<td>Stop at 245</td>
<td>+</td>
</tr>
<tr>
<td>KS-198</td>
<td>OS</td>
<td>7</td>
<td>241</td>
<td>Yes</td>
<td>TCC to TAC</td>
<td>Ser to Tyr</td>
<td>-</td>
</tr>
<tr>
<td>KS-208</td>
<td>OS</td>
<td>7</td>
<td>244</td>
<td>Yes</td>
<td>GGC to TGC</td>
<td>Gly to Val</td>
<td>-</td>
</tr>
<tr>
<td>KS-283</td>
<td>CS</td>
<td>7</td>
<td>249</td>
<td>Yes</td>
<td>AGG to ACG</td>
<td>Arg to Thr</td>
<td>+</td>
</tr>
<tr>
<td>KS-154</td>
<td>OS</td>
<td>7</td>
<td>250</td>
<td>Yes</td>
<td>CCC to CTC</td>
<td>Pro to Leu</td>
<td>-</td>
</tr>
<tr>
<td>258-OT</td>
<td>OS</td>
<td>7</td>
<td>259</td>
<td>No</td>
<td>GAC to GTC</td>
<td>Asp to Val</td>
<td>-</td>
</tr>
<tr>
<td>KS-96</td>
<td>MFH</td>
<td>8</td>
<td>273</td>
<td>Yes</td>
<td>CGT to CAT</td>
<td>Arg to His</td>
<td>+</td>
</tr>
<tr>
<td>KS-241</td>
<td>OS</td>
<td>8</td>
<td>273</td>
<td>Yes</td>
<td>CGT to CAT</td>
<td>Arg to His</td>
<td>+</td>
</tr>
<tr>
<td>KS-81</td>
<td>OS</td>
<td>8</td>
<td>281</td>
<td>Yes</td>
<td>GAC to CAC</td>
<td>Asp to His</td>
<td>-</td>
</tr>
<tr>
<td>KS-103</td>
<td>OS</td>
<td>8</td>
<td>281</td>
<td>Yes</td>
<td>GAC to AAC</td>
<td>Asp to Asn</td>
<td>-</td>
</tr>
<tr>
<td>KS-197</td>
<td>OS</td>
<td>9</td>
<td>Splice acceptor site</td>
<td>No</td>
<td>aaTG to gtCG</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>KS-39</td>
<td>OS</td>
<td>9</td>
<td>316–7</td>
<td>No</td>
<td>1 base pair insertion</td>
<td>Stop at 336</td>
<td>+</td>
</tr>
<tr>
<td>KS-107</td>
<td>LS</td>
<td>9</td>
<td>Splice donor site</td>
<td>No</td>
<td>AGt to AGt</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* OS, osteosarcoma; MFH, malignant fibrous histiocytoma; LS, liposarcoma.

monkey, rat, and mouse (23). Therefore, all 13 of the missense mutations we found occurred at highly conserved residues of the p53 gene, whereas nonsense mutations appeared to occur at random.

Of the 21 point mutations we found, 17 are single base substitutions. The nature of these base substitutions is shown in Table 4; 8 were transitions (4 of which occurred at CpG dinucleotides), and 9 were transversions. Although the total number of mutations is small, our data show no clear hotspots and no preponderance of one particular type of mutation. Two instances of a single base deletion and one instance of a single base insertion occurred in strings of G or C nucleotides (KS-146, deletion of one C from a string of 4 C's; KS-131, deletion of one C after a 3-string of G's; KS-39, insertion of one C in a string of 4 C's). Finally, a 4-base pair deletion was found in a region within a direct repeat (KS-140, GCTCTGACTG to GCTCTG).

Clinical Phenotypes of Osteosarcomas with p53 Gene Mutations. Of all osteosarcoma patients in this study, clinical follow-up of 2 or more years after initial treatment is available for only 36 patients. Lung metastases developed in 21 of 36 cases; 10 of these (48%) occurred in patients whose primary tumors carried one or more p53 gene mutations. Fifteen osteosarcoma patients showed no evidence of disease during the 2 years that...
p53 GENE MUTATIONS IN SARCOMAS

Fig. 2. a, SSCP analysis of exon 7 of the p53 gene in a patient with osteosarcoma (KS-154). Control and KS-154 are DNAs from leukocytes, and KS-154-T is DNA from tumor tissues. b, SSCP analysis of exon 9 of the p53 gene in a patient with malignant fibrous histiocytoma (KS-39). c, SSCP analysis of exon 9 of the p53 gene in a patient with liposarcoma (KS-107). Arrows, variant bands.

Fig. 3. Direct genomic sequence analysis of the p53 gene in patients shown in Fig. 2. a, homozygous C to T transition at codon 250 in exon 7. b, homozygous insertion of one C in a string of 4 Cs spanning codons 316-317 in exon 9. c, heterozygous G to T transversion at the splice donor site of intron 9.
followed their initial treatment. p53 gene mutations were identified in 7 patients from this group (47%). Therefore, no definite association was observed between p53 gene mutation and the incidence of lung metastasis in the 36 patients for whom a 24-month clinical follow-up was available.

Association of p53 Gene Mutations with Allele Loss on 17p. Allelic deletions on 17p were previously found in approximately 70% of osteosarcoma tumors, and the common region of allele loss was assigned to 17p13, where the p53 gene is located (19, 24). These data suggest that allelic deletions on 17p occur as a second event following an initial mutation on the homologous copy of p53 gene. Of 76 cases of osteosarcoma analyzed in this study, 57 cases were informative (constitutionally heterozygous) for the analysis of allele loss on 17p (Table 5). In 39 of 57 cases (68%), tumor cells lost one allele at one or more polymorphic loci on 17p. Alterations in the p53 gene, including either gross rearrangements or point mutations, were identified in 51% (20 of 39) of tumors with allele loss on 17p. No detectable mutations were found in the remaining 49% of tumors with allele loss on 17p. Genetic alterations of the p53 gene were found in 50% (9 of 18) of tumors that were informative for 17p markers and which retained both alleles at the p53 locus.

DISCUSSION

A number of studies have been reported concerning the incidence of p53 gene mutations in human cancers, allowing a comparison of the mutation spectrum we found in sarcomas with that seen in other types of malignant tumors. Our study revealed a relatively high incidence (21 of 126) of gross rearrangements of the p53 gene in sarcomas. This is consistent with previous reports describing p53 gene mutations in sarcomas (15, 25–27). Point mutations of the p53 gene have also been found with a high frequency in various other types of cancers. However, only chronic myelogenous leukemia at blast crisis showed an equivalent frequency of gross rearrangements of the p53 gene (28, 29). In these previous studies, the majority of rearrangements in both sarcomas and chronic myelogenous leukemia were found to occur in intron 1, and tumors with rearrangements were shown to have no detectable mRNA of the p53 gene (27–29), indicating that rearrangements of the p53 gene usually cause loss-of-function mutations. A similar pattern of rearrangements was observed in our study, although a precise analysis of the rearrangement could not be performed in many cases because DNA from the primary tumor was limited.

The fraction of sarcomas with loss-of-function mutations in the p53 gene increases significantly if point mutations leading to nonsense errors are included. Of the 21 point mutations we found, 6 mutations create a premature stop codon downstream, leading to the truncation of important regions known to complex with viral antigens or wild-type p53 protein (30, 31). Although it is likely that the two mutations found at splicing sites of exon 9 are also loss-of-function mutations, neither RNA nor protein studies could be carried out on these tumors. Therefore, at least 27 (21 rearrangements and 6 nonsense mutations) of 42 (64%) of the p53 gene mutations we identified in 127 sarcomas can be regarded as loss-of-function mutations.

Interestingly, we found a similar mutation spectrum among germ-line p53 gene mutations in sarcoma patients. Although the number of cases was much smaller, 4 of 8 patients with germ-line p53 gene mutations carried loss-of-function mutations (32). This predominance of loss-of-function mutations in sarcomas is considerably different from the mutation spectrum reported for other types of cancer such as colorectal carcinomas or non-small cell lung cancer, where missense errors accounted for 94% (30 of 32 cases) or 91% (21 of 23) of all mutations, respectively (10, 12). Since the number of potential targets for nonsense mutations is theoretically much greater than that for functionally significant missense mutations, it is reasonable to expect that the spectrum of single base substitutions would be broad in tumors with a tendency toward loss-of-function mutations. Indeed, the mutations we observed in 127 sarcomas spanned a wide region of the p53 gene, from codons 46 to 316. Our findings emphasize the importance of screening, at a minimum, the entire p53 coding region if an accurate evaluation of the spectrum of p53 gene mutations is sought.

Our detection of a disproportionately low frequency of p53 gene mutations among tumors with allele loss on 17p is quite interesting with respect to the “two-hit hypothesis” of tumor suppressor gene action. By comparison, a study of p53 mutation and allelic loss on 17p in colorectal carcinoma has shown that 86% of tumors with 17p allelic loss carried a mutation in the p53 gene (10). Several explanations could account for the disparate findings in these two studies. First, studies that do not include a detailed analysis of the promoter and exon 1 might underestimate the overall frequency of p53 mutations by failing to detect mutations in regulatory regions, as have been described for another tumor suppressor, the retinoblastoma gene (33). Since the promoter region of the p53 gene was not analyzed in our study, the possibility exists that we missed such mutations; hence, it is possible that most of the tumors with allele loss in our study do carry p53 mutations, but they went undetected. A second possibility is that the p53 gene was not
mutated in some tumors with allelic losses on 17p. The presence of another tumor suppressor gene on 17p telomeric to the p53 locus has been postulated in studies of breast cancer (34) and hepatocellular carcinoma (35) and could account for our data. Finally, a third possibility is that allelic loss at the p53 locus might itself have oncogenic potential in some target cells and would thus precede detectable mutations at the remaining locus in these cases. In colorectal carcinomas, missense mutations are supposed to precede allelic deletions; the mutant proteins are assumed to create a growth advantage through a "dominant negative" effect. Therefore, cells with one mutant allele may lose the remaining normal allele during a phase of rapid proliferation (10). Thus, a simple quantitative decrease of the p53 gene product might be enough to endow cells with a growth advantage in a tissue-specific manner. The fact that approximately one-half of tumors without allelic deletions on 17p in this study have an alteration of the p53 gene may support this model. This hypothesis would be more compelling if expression of the two alleles of the p53 gene is not equivalent in all cells, as has been shown for other genes (36, 37). A precise analysis of the expression of the p53 gene in tumors without detectable mutations would further our understanding of this issue.

ACKNOWLEDGMENTS

We thank Dr. T. Dryja for his cooperation; Drs. N. Takada, T. Umeda, N. Kawaguchi, Y. Kaneko, I. Sprio, A. Rosenberg, and H. Halprin for providing materials and clinical information; Dr. Y. Nakamura for polyomavirus on 17p; Drs. H. Koeffler and C. Miller for a cDNA clone of the p53 gene; Drs. L. Crawford and S. Tuck for a genomic clone containing the p53 gene; and Drs. B. Ludeke, R. Chung, J. Whaley, G. Cowley, and B. Seizinger for technical advice.

REFERENCES

Mutation Spectrum of the \textit{p53} Gene in Bone and Soft Tissue Sarcomas

Junya Toguchida, Toshikazu Yamaguchi, Bruce Ritchie, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/22/6194

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.