Frequent p53 Gene Mutations in Soft Tissue Sarcomas Arising in Burn Scar

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Squamous cell carcinoma (SCC) is the commonest malignancy that arises in burn scars, which frequently contain p53 mutations. Soft tissue sarcoma (STS) also develops, though less frequently, in burn scars. p53 gene mutations were analyzed in paraffin-embedded specimens from 5 patients with STS (4 males and 1 female) that had arisen in a burn scar, by means of polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) followed by direct sequencing. Age at burn injury ranged from 2 to 10 (median 3) years, and STS developed with a latent period ranging from 29 to 79 (median 60) years. Histologically, all were malignant fibrous histiocytoma. The PCR-SSCP revealed aberrant bands in 4 (80%) of 5 cases. Direct sequencing revealed a total of 11 mutations in these 4 cases: 1 case had a single mutation, 1 had 2 mutations, and 2 had 4 mutations. Every tumor had at least 1 mutation that changed an amino acid, which may have provided the selection pressure for expansion. Thus, there is a high frequency of p53 gene mutations in STS appearing in burn scars. p53 mutations were also frequent in pyothorax-associated lymphoma (PAL), a lymphoma that develops in patients with long-standing pyothorax, so p53 mutations might be frequent in malignancies that develop in chronic inflammatory sites.

Key words:    Soft tissue sarcoma — Burn scar — p53 mutation — Polymerase chain reaction — Single strand conformation polymorphism

PATIENTS AND METHODS

A review of the literature published in Japan provided 4 cases with malignant fibrous histiocytoma (MFH) and 1 case with an unclassifiable sarcoma in a burn scar8–12) (Table I). These patients had been admitted to hospitals in Japan during 1984 to 1989. The criteria for case selection were as follows: 1) prior history of thermal injury; 2) development of STS within a burn scar. The latent period between the thermal injury and development of STS ranged from 29 to 79 (median 60) years. Histologic specimens were obtained from the primary tumor in 3 cases and metastatic tumor in 2 (Fig. 1). In 2 (cases 1, 2) of these 5 cases, SCC also arose near the STS: SCC arose 8 years after surgery for MFH in case 1 and SCC and MFH developed simultaneously but at separate locations in case 2. In 2 metastatic cases, specimens from metastatic skin and lung lesions (case 4) or lymph nodal lesion (case 3) were available, but those from the primary tumor were not. All of the histologic specimens were fixed in 10% formalin and routinely processed for paraffin-embedding. Histologic sections, cut at 5 µm, were stained with hematoxylin and eosin. Histologic findings and brief clinical data of these patients are summarized in Table I. All tumors at the primary site presented as a soft tissue mass covered with intact skin. Histologic diagnosis of MFH...
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was made based on the criteria of Enzinger and Weiss.\(^2\) If diagnosis was problematic on purely morphological grounds, immunohistochemical methods using primary antibodies including cytokeratin, α-1-antitrypsin and lysozyme were employed. All cases were negative for cytokeratin, but positive for α-1-antitrypsin and/or lysozyme.

**Detection of p53 mutations** DNA was extracted from the paraffin-embedded tissue using chelating resin (Sigma, St. Louis, MO).\(^3\) The PCR primer pairs for the amplification of the p53 gene exons 5–8 were: a) 5′-GTACTC-CCCTGCCCCTCAACA-3′ and 5′-CTCACCATCGGTATCTGAGCA-3′ for exon 5; b) 5′-TGCTCTTAAGGCTCTGGGCCCC-3′ and 5′-CAGACCTCAGGCGGCTCATA-3′ for exon 6; c) 5′-TAGGTGCTGGCTGACTGACCTGC-3′ and 5′-TGACCTGGAGTCTTCCAGTGT-3′ for exon 7; d) 5′-AGGTGTAATCCTACTGGGACGG-3′ and 5′-ACCTAGCTTAGTGCTCCTG-3′ for exon 8. PCR amplification and nonradioactive SSCP (cold SSCP) were carried out to detect mutations as described previously.\(^14, 15\) The mutated SSCP bands were extracted from the gel and reamplified by PCR for 20 cycles to enrich the mutated alleles. Sequencing was performed by the dideoxy chain termination method using the AmpliTaq FS cycle-sequencing kit (Perkin-Elmer Corp., Foster City, CA). Sequencing primers were the same as those used for PCR. Cycle sequencing was performed based on the protocol, i.e., 30 cycles of denaturation (95°C, 30 s), annealing (52°C, 30 s), and extension (72°C, 4 min) followed by 20°C after the final cycle. After ethanol precipitation, the samples were analyzed on a Genetic Analyzer (ABI PRISM 310, Perkin-Elmer Corp.). PCR-SSCP analyses and sequencing of mutated bands were repeated 3 times for each sample to rule out the possibility of contamination and PCR fidelity artifacts.

As control, we also examined p53 mutations in 20 cases of sporadic STS (19 MFH and 1 leiomyosarcoma).

### RESULTS

Upon PCR-SSCP analysis, the samples that showed electrophoretic mobility shifts compared with the control DNA were considered to contain a mutant p53 gene.
A:T transitions were detected in 2 metastatic tumors; codon 238 (TGT to GTA), codon 233 (CAC to CAT), and codon 138 (GCC to GAC) in the skin and codon 139 (ACT) in SCC (case 2).

The PCR-SSCP followed by direct sequencing showed aberrant mobility shifts of bands in 4 (80%) of 5 cases with STS that developed in burn scars. Most of the previous studies on p53 mutation within exons 5 to 8 in sporadic STS found a frequency of 11–20%, close to that in our control cases of sporadic STS (20%). The p53 mutation frequency of sporadic MFH was reported to be 12%. The difference in frequency of p53 gene mutations between STS developing in burn scars and sporadic STS was statistically significant ($\chi^2$ test; $P<0.05$). The occurrence of point mutations at multiple sites (11 point mutations/4 cases) was also characteristic in our cases. These findings highlighted the presence of an extraordinarily high frequency of p53 gene mutations in STS developing in burn scars.

In various human cancers, more than 80% of p53 mutations are single base substitutions frequently affecting codon 245, 248, 249, 273 or 282, i.e., mutational ‘hot spots.’ In the present series, every tumor had at least 1 mutation that changed an amino acid, although these mutations were not found in the so-called ‘hot spots.’ Nevertheless, all of 6 missense mutations occurred in highly conserved regions (II and IV), which are known to have an important role in the binding of p53-responsive elements which function as transcriptional activators.

p53 is referred to as the “guardian of genome” because it affects cell cycle arrest in the G1 phase in response to DNA damage. Loss of this checkpoint control could result in replication of damaged DNA, and the generation of genomic instability in affected cells.

In burn scars with eczematous dermatitis, nitric oxide and other oxygen radicals are produced by inflammatory cells, and may cause gene damage. Thus, p53 gene mutations might well be frequent in malignancies developing in chronic inflammatory sites. Frequent p53 mutations in pyothorax-associated lymphoma (PAL) were recently reported. PAL develops in patients with long-standing pyothorax (33 years on average) resulting from artificial pneumothorax for the treatment of pulmonary tuberculosis or tuberculous pleuritis. The frequency of p53 mutations in PAL was also high (67%), and characteristically occurred at dipyrimidine sites in 77% of the p53 mutation-positive cases. Mutation at the dipyrimidine sites was found in a half of the current cases. G:C→A:T transitions were found in about 70% of STS in burn scars and 90% of PAL cases, while 1 of 4 point mutations in the sporadic STS cases was a G:C→A:T transition. These findings show similarities in the patterns of p53 mutation between PAL and STS developing in burn scars.

ACKNOWLEDGMENTS

The authors thank the following doctors for providing clinical information and histologic materials: Drs. S. Sugiyama (Sapporo Medical Univ.), Y. Yamamoto, T. Moriki (Kochi Medical Univ.), N. Ishii, and M. Hara (Yokohama City Univ.).

(Received November 2, 1998/Revised December 28, 1998/ Accepted January 11, 1999)
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