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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

Squamous cell carcinoma (SCC) and also soft tissue sarcoma (STS), though much less frequently, arise in thermal injury (burn) scars.[1)] Patients usually have a history of extensive burns during childhood, and STS or other malignancies develop after a long latent period.[2)] Extensive burn scarring is frequently accompanied with eczematous change due to chronic inflammation. Accumulating evidence suggests that inflammatory cells can induce genotoxic effects including DNA strand breaks, sister chromatid exchange,[3)] mutation,[4)] and neoplastic transformation.[5)]

The tumor suppressor gene *p53* plays a central role in the cellular response to DNA damage, through causing cell cycle arrest in the G1 phase or apoptosis.[6, 7)] Thus, in the current study, *p53* gene mutations in 5 cases of STS that developed in burn scars were examined by exon-specific polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis of exons 5–8, followed by direct sequencing.

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Abbreviations: STS, soft tissue sarcomas; MFH, malignant fibrous histiocytoma; PCR, polymerase chain reaction; SSCP, single strand conformation polymorphism; SCC, squamous cell carcinoma; PAL, pyothorax-associated lymphoma.

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 scar[8–12)] (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

Table I. Clinical Findings and *p53* Abnormalities by PCR-SSCP Analysis in 5 Patients with Soft Tissue Sarcoma

Case	Sex	Age (years)	Burn site	Latency (years)	Distant metastasis	Coexistence of SCC	Histology of sarcoma	*p53* mutation				
								Exon	Conserved region	Codon	Nucleotide	Amino acid
1	M	62	Face	60	No	Yes	MFH					
2	F	84	Buttock	79	No	Yes	MFH	5	—	152	CCG/CCA	Pro/Pro
								5	III	173	GTG/GTA	Val/Val
								7	—	233	CAC/CAT	His/His
								7	IV	250	CCC/TCC	Pro/Ser
3	M	32	Elbow	29	Yes	No	MFH	7	IV	254	ATC/AAC	Ile/Asn
								8	V	277	TGT/TGC	Cys/Cys
4	M	48	Knee	38	Yes	No	MFH	7	IV	238	TGT/CGT	Cys/Arg
5	M	64	Head	62	No	No	MFH	5	II	142	CCT/TCT	Pro/Ser
								5	—	145	CTG/TTG	Leu/Leu
								7	IV	244	GGC/AGC	Gly/Ser
								7	IV	249	AGG/AGA	Arg/Arg

SCC, squamous cell carcinoma; MFH, malignant fibrous histiocytoma.

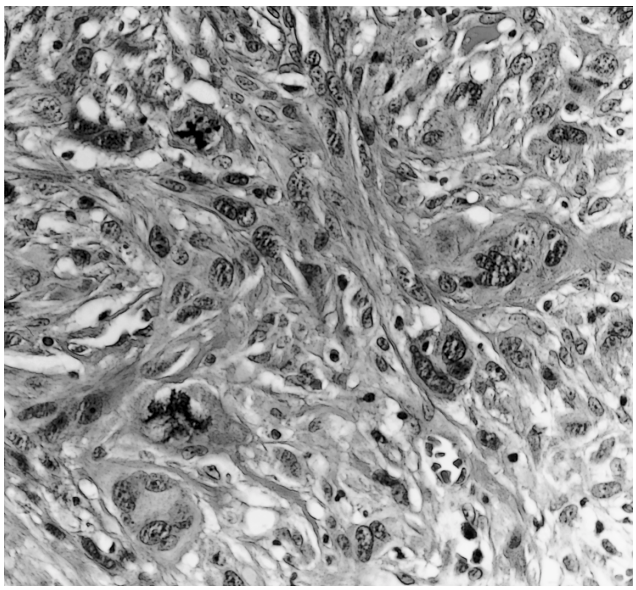

Fig. 1. Malignant fibrous histiocytoma with pleomorphic appearance (HE, ×450).

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).[13] The PCR primer pairs for the amplification of the *p53* gene exons 5–8 were: a) 5′-GTACTCCCCTGCCCTCAACA-3′ and 5′-CTCACCATCGCTATCTGAGCA-3′ for exon 5; b) 5′-TTGCTCTTAGGTCTGGCCCC-3′ and 5′-CAGACCTCAGGCGGCTCATA-3′ for exon 6; c) 5′-TAGGTTGGCTCTGACTGTACC-3′ and 5′-TGACCTGGAGTCTTCCAGTGT-3′ for exon 7; d) 5′-AGTGGTAATCCTACTGGGACGG-3′ and 5′-ACCTAGCTTAGTGCTCCCTG-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.

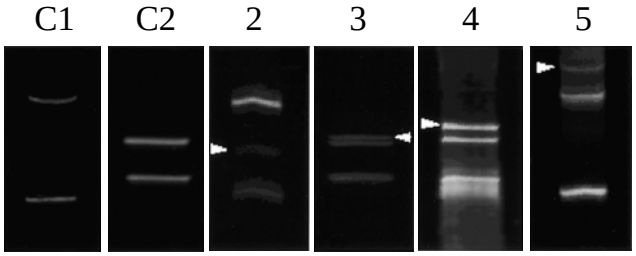

Fig. 2. Nonradioactive SSCP. Wild-type bands in control cases were seen in C1 (exon 5) and C2 (exon 7). Aberrant bands (arrowheads) were observed above or below the upper wild-type band; direct sequencing from each aberrant band revealed the point mutations (cases 2, 3, 4, 5).

Aberrant bands were observed in 4 of 5 cases with STS; 2 cases in exon 5, 4 in exon 7, and 1 in exon 8. 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 (Fig. 2). Among these 11 mutations, 5 were missense mutations leading to amino acid substitutions and 6 silent mutations with no amino acid changes. All these 4 cases had at least 1 mutation that changed an amino acid (Table I). All of the 5 missense mutations involved the highly conserved domains II–V, but did not occur in the previously reported 'hot-spot.' Eight of the 11 substitutions were G:C→A:T transitions, and G:C→A:T transitions at dipyrimidine sites were found in 2 (40%) of 5 cases. Two mutations were A:T→G:C transitions, and 1 was an A:T→T:A transversion. In 1 metastatic case (case 4), different mutations were detected in 2 metastatic tumors; codon 238 (TGT to CGT) in the skin and codon 138 (GCC to GAC) in the lung lesion. The mutational patterns in the SCC from 2 cases differed from those in the adjoining STS; no mutation in STS but mutation in codon 180 (GAG to GAT) in SCC (case 1); mutations in codon 152 (CCG to CCA), codon 173 (GTG to GTA), codon 233 (CAC to CAT), and codon 250 (CCC to TCC) in STS and codon 256 (ACA to ACT) in SCC (case 2).

Four (20%) of 20 cases with sporadic STS showed point mutation due to single base substitution (codon 139; AAG→AAC, 151; CCC→GCC, 154; GGC→GGT, 249; AGG→AGA).

DISCUSSION

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%,[16–18] which was close to that in our control cases of sporadic STS (20%). The *p53* mutation frequency of sporadic MFH was reported to be 12%.[19] The difference in frequency of *p53* gene mutations between STS developing in burn scars and sporadic STS was statistically significant (χ^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.'[6] 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.[20] *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.[21] 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.

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REFERENCES

1) Lever, W. F. "Histopathology of the Skin," 7th Ed., pp. 552–553 (1989). Lippincott-Raven, Philadelphia, NY.
2) Enzinger, F. M. and Weiss, S. W. "Soft Tissue Tumors," 3rd Ed., p. 281 (1995). Mosby, St. Louis, MO.
3) Weitberg, A. B. Effect of combinations of antioxidants on phagocyte-induced sister chromatid exchanges. *Mutat. Res.*, **224**, 1–4 (1989).
4) Weitzman, S. A. and Stossel, T. P. Mutation caused by human phagocytes. *Science*, **212**, 546–547 (1981).
5) Wei, H. and Frenkel, K. Suppression of tumor promotor-induced oxidative events and DNA damage *in vivo* by sarcophytol A : a possible mechanism of antipromotion. *Cancer Res.*, **52**, 2298–2303 (1992).
6) Hollstein, M., Sidransky, D., Vogelstein, B. and Harris, S. R. p53 mutations in human cancers. *Science*, **253**, 49–53 (1991).
7) El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parson, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W. and Vogelstein, B. WAF1, a potential mediator of p53 tumor suppression. *Cell*, **75**, 817–825 (1993).
8) Sato, N., Takahashi, N., Saito, K. and Li, R. A case of malignant fibrous histiocytoma on burn scar, and review of literature. *Jpn. J. Clin. Dermatol.*, **43**, 851 (1989) (in Japanese).
9) Obara, A., Arata, J. and Yamamoto, Y. Coexistence of squamous cell carcinoma and malignant fibrous histiocytoma on burn scar. *Jpn. J. Clin. Dermatol.*, **38**, 443–446 (1984) (in Japanese).
10) Akutsu, Y., Yamana, K., Kawamura, M. and Sugiyama, S. Malignant fibrous histiocytoma arising in burn scar. *Jpn. J. Dermatol.*, **29**, 201–205 (1987) (in Japanese).
11) Takahashi, Y., Uchiyama, H., Nakajima, H., Nagai, R., Hayashi, M. and Yoshida, S. Malignant fibrous histiocytoma arising from a burn scar. *Jpn. J. Clin. Dermatol.*, **96**, 867 (1986) (in Japanese).
12) Yamamura, T., Aozasa, K., Honda, T., Takada, A., Maeda, M. and Sano, S. Malignant fibrous histiocytoma developing in a burn scar. *Br. J. Dermatol.*, **110**, 725–730 (1984).
13) Naka, N., Tomita, Y., Nakanishi, H., Araki, N., Hongyo, T., Ochi, T. and Aozasa, K. Mutations of p53 tumor-suppressor gene in angiosarcoma. *Int. J. Cancer*, **71**, 952–955 (1997).
14) D'Aquil, R. T., Bechtel, L. J., Videler, J. A., Eron, J. J., Gorczyc, P. and Kaplan, J. C. Maximizing sensitivity and specificity of PCR by pre-amplification heating. *Nucleic Acids Res.*, **9**, 3749 (1991).
15) Hongyo, T., Buzard, G. S., Palli, D., Weghorst, C. M., Amorosi, A. Galli, M., Caporaso, N. E., Fraumeni, J. F. and Rice, J. M. Mutations of K-ras and p53 genes in gastric adenocarcinoma from a high-incidence around Florence, Italy. *Cancer Res.*, **55**, 2665–2672 (1995).
16) Toguchida, J., Yamaguchi, T., Ritchie, B., Beauchamp, R. L., Dayton, S. H., Herrera, G. E., Yamamuro, T., Kotoura, Y., Sasaki, M. S., Little, J. B., Weichsel-Baum, R. R., Ishizaki, K. and Yandell, D. W. Mutation spectrum of the p53 gene in bone and soft tissue sarcomas. *Cancer Res.*, **52**, 6194–6199 (1992).
17) Helge, T., Axel, M. and Peter, W. Prognosis is correlated with p53 mutation type for soft tissue sarcoma patients. *Cancer Res.*, **56**, 4134–4136 (1996).
18) Castresana, J. S., Rubio, M. P., Gomez, L., Kreicbergs, A., Zetterberg, A. and Barrios, C. Detection of P53 gene mutations in human sarcomas. *Eur. J. Cancer*, **31**, 735–738 (1995).
19) Reid, A. H., Tsai, M. M., Venzon, D. J., Wright, C. F., Lack, E. E. and O'Leary, T. J. MDM2 amplification, P53 mutation, and accumulation of the P53 gene product in malignant fibrous histiocytoma. *Diagn. Mol. Pathol.*, **5**, 65–73 (1996).
20) Wang, Y. and Prives, C. Increased and altered DNA binding of human p53 by S and G2/M but not G1 cyclin-dependent kinases. *Nature*, **376**, 88–91 (1995).
21) Hongyo, T., Kurooka, M., Taniguchi, E., Iuchi, K., Nakajima, Y., Aozasa, K. and Nomura, T. Frequent p53 mutations at dipyrimidine sites in patients with pyothorax-associated lymphoma. *Cancer Res.*, **58**, 1105–1107 (1998).