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Clinical Observations

DETECTION OF POINT MUTATION OF p53 GENE BY SILVER STAINING PCR-SSCP IN PARAFFIN-EMBEDDED MALIGNANT FIBROUS HISTIOCYTOMA

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Silver staining PCR-SSCP method was used to detect point mutation of p53 gene in paraffin-embedded malignant fibrous histiocytoma (MFH) tissues. The abnormal shifting of the single-stranded DNA (ssDNA) was identified in 9 out of 16 cases (56.3%). The positive figure of SSCP was 1, 4, 4, 3 in exon 5, 6, 7, 8, respectively. The mutant of p53 protein was detected by microwave oven treatment and ABC immunohistochemistry. Positive nuclear staining was observed in 10 cases (62.5%). The positive coincidence rate was 90.0% between SSCP and p53 protein expression. The mutation of p53 gene was not correlated with the subtypes of MFH. Our results indicate that detection of point mutations with silver staining PCR-SSCP is convenient, rapid and reliable in the screening of point mutation of genes.

Key words: Histiocytoma, Protein p53, Immunohistochemistry, Polymerase chain reaction, Mutation.

The p53 gene, located on chromosome 17p, encodes a 53-kDs DNA binding nuclear phosphoprotein with a short half-life that negatively regulates cell growth and proliferation, and its alteration or loss is thought to deprive the cell of these inhibitory signals.[1] Mutations of tumor suppressor gene p53 have been demonstrated for more than 50% of all tumors, thus constituting the most frequent genetic alteration within the framework of the tumor process.[2]

In general, there is a less frequent occurrence of p53 mutations in soft tissue sarcomas (STS) than in carcinomas. Comparing the number of 92 mutations for soft tissue tumors recorded up to now with the total number of more than 3500 entries in the p53 mutation database, STS make up less than 3% of all mutations registered.[3,4]

The aim of this study, was to explore the presence and pattern of mutations of the p53 gene and of p53 protein immunohistochemical expression in a panel of malignant fibrous histoicytoma (MFH) and to compare the results with clinicopathological data.

MATERIALS AND METHODS

Tumor Samples

A total of 16 various types of MFH were analysed. The histological diagnoses of these cases are listed in Table 1.

DNA Isolation and Polymerase Chain Reaction

The DNA from paraffin sections was isolated according to a standard method.[5] Through the polymerase chain reaction (PCR), exons 5-8 of the

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p53 gene were amplified. We used four pair of primers described by Ruggeri et al.[6] Polymerase chain reaction conditions were as follows: predenaturation at 97 ℃ for 5 min. and 35 cycles of denaturation at95 ℃ for 30 sec., annealing for 1 min. at 55 ℃ (exons 5, 7–8), 68 ℃ (exon 6), and DNA synthesis for 1 min. at 72 ℃; postsynthesis for 10 min. at 72 ℃ and storage of the PCR products at 4 ℃.

Table 1. p53 expression in 16 cases of MFH detected by immunohistochemistry and comparison with SSCP analysis

Criterion	Number of cases	SSCP n	Positive %	Immunohistochemistry*			
				–	+	++	%
MFH	16	9	56.3	6	4	6	62.5
Common type	11	6		5	2	4	
Mucous type	2	1		1	1	0	
Inflammation type	2	1		0	1	1	
Giant cell type	1	1		0	0	1	
Metastasis							
Negative	14	7		6	3	5	
Positive	2	2		0	1	1	

*–: <10%; –: 10–50%; ++: >50% immunohistochemically positive cancer cells.

Single Strand Conformation Polymorphism Analysis

As a screen for p53 mutation, a silver-staining single strand conformation polymorphism (SSCP) analysis was performed. Ten to 15 μl (approximately 1.5 μg) of PCR product were denatured for 6 min. at 96 ℃ in SSCP buffer (80% formamide, 20 mM ethylene diamine tetraacetic acid, 0.05% xylene cyanol FF and 0.05% bromphenol blue) and immediately cooled on ice. The samples were loaded onto a 6% or 10% nondenaturing tris-borate ethylenediamine tetraacetic acid buffer gel and run at room temperature for 5– 6 h. (10 mA). Staining of the single-and double-stranded DNA was performed according to a silver staining protocol.[7]

Immunohistochemistry

The 5 μm sections were deparaffinized in xylene and dehydrated with graded ethanol. Endogenous peroxidase activity and nonspecific binding were blocked by treatment with 3% hydrogen peroxide for 10 min. and treatment with microwave for 15 min., respectively. Then treated with 3% normal horse serum for 10 min. at 37 ℃. Monoclonal antibody (DO-1, DAKO) was applied to the sections at a dilution of 1:80 and incubated for 12 h. at 4 ℃. Immunostaining was performed using abiotinylated antimouse antibody kit (Vector Lab.) and diaminobenzidine (Sigma) (0.6 mg/mL) with 3% of hydrogen peroxide. The positive control specimen was obtained from a known p53-mutated larynx carcinoma and was in each assay. Phosphate-buffered saline was used in place of primary antibody as a negative control medium for immunostaining.

Nuclear staining of tumor cells was considered positive for p53 expression. Differences in p53 expression were evaluated with respect to clinical data and the histological type by the x^2 test.

RESULTS

p53 Mutations in Exons 5–8

Sixteen tumor samples were examined for p53 mutations by a silver-staining PCR-SSCP analysis. Abnormalities in the single-stranded DNA pattern, indicating p53 mutations, were determined for 9 samples (56.3%) (Figure 1). The positive figure of SSCP was 1, 4, 4, 3 in 5, 6, 7, and 8, respectively.

p53 gene mutation was not correlated with the subtypes of the MFH (Table 1).

p53 Immunoreactivities

Ten samples (62.5%) of MFH examined were immunohistochemically p53 positive. There was not correlation between p53 protein expression and the subtypes of MFH. The positive coincidence rate between p53 gene mutation and protein expression was 90.0%, which can reflect the p53 gene mutation approximately.

Among the neoplasms with metastasis, the mutation rate of p53 gene and positive rate of p53 protein expression were much higher than these without metastasis and they were almost the same in the primary neoplasms.

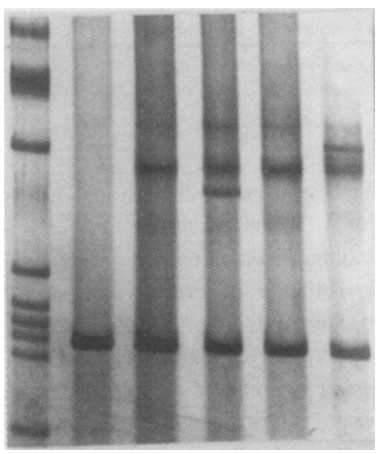

Fig. 1. Polymerase chain reaction-single strand conformation polymorphism analysis of p53 gene (exon 6) in MFH tumor samples. Lanes are 1-6 from left to right. Lane 1 is DNA molecular weight marker pBR322/Hae III. Lane 2 is nondenature dsDNA. Normal ssDNA pattern for control (lane 3) and one tumor sample without a mutation (lane 5). Shift in ssDNA pattern for tumor samples (lane 4, 6) indicates a p53 mutation. (ssDNA: single strand DNA, dsDNA: double strand DAN).

DISCUSSION

The important relationships between p53 gene mutation and carcinogenesis has been proved in most of the epithelial neoplasms. Mesenchymal neoplasms, especially its malignant tumor — sarcomas, are the second common malignant neoplasms of human being. Owing to their complicated, diversified, and somewhat similar pathological morphology, as well as high tendency of metastasis due to rich in vascularity, the pathological diagnosis and differential diagnosis, judgement of their malignancy, metastatic potentiality and prognosis are very difficult. From molecular level to study their biologic behavior maybe will solve some of the problems.

In the current study, MFH were examined for the presence of p53 mutations and for the character of their p53 immunoreactivity. Through a silver-staining PCR-SSCP method, 16 MFH samples were analyzed for possible DNA alterations at the p53 gene locus.

The frequency of p53 positive cases in this study (56.3%) is not the same as that of two previous reports.[8,9] Taubert et al. reported that 21 out of 30 (70%) MFHs showed p53-positive cells and Wadayama et al. reported that p53 immunoreactivity was found in 4 out of 11 (36.4%) of MFHs. The difference in frequency may be due to the difference of the antibodies used. Alternatively, it might be caused by the difference of types and numbers of tumors in each study.

We found that 1 of 16 cases with no apparent DNA alteration at the p53 locus showed weak but positive staining for the p53 protein. This tumor may posses some types of mutations, especially missense mutations, which failed to be detected in the initial screening of PCR-SSCP analysis, or were located outside of the analysed regions. The positive coincidence rate between p53 gene mutation and protein expression was 90.0%. Our results were coincidence with other cancers such as colon carcinomas where more than 90% of the mutations were shown to produce mutant p53 protein.[10] Our data suggest that there is a good overlap between the presence of a mutation at the DNA level and positive immunostatining in MFHs since 1/10 samples scored negative upon PCR-SSCP analysis. If, according to the present data, positive immunostaining often corresponds to the presence of a mutation at the genetic level, the reverse is not always true.

The p53 mutations we identified for malignant fibrous histiocytoma supplement the mutation spectrum that has been found up to now for soft tissue sarcomas, and the findings encourage the search for further mutations, to examine their characteristics and to explain correlations with the clinical process.

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