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p53 gene mutation analysis in porokeratosis and porokeratosis-associated squamous cell carcinoma

Yoshiro Ninomiya[a], Yoshio Urano[a,*], Katsuhiko Yoshimoto[b], Hiroyuki Iwahana[b], Shiro Sasaki[a], Seiji Arase[a], Mitsuo Itakura[b]

[a]*Department of Dermatology, School of Medicine, The University of Tokushima, Kuramoto-cho, Tokushima 770, Japan*
[b]*Otsuka Department of Clinical and Molecular Nutrition, School of Medicine, The University of Tokushima, Kuramoto-cho, Tokushima 770, Japan*

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Abstract

In this and previous studies, we have shown p53 overexpression immunohistochemically in 14 of 17 porokeratotic specimens obtained from 14 lesions of nine cases, and in all six specimens of squamous cell carcinoma (SCC) arising on porokeratotic lesions of two cases. We screened mutations in exons 5 to 10 of the p53 gene in all these specimens by polymerase chain reaction-single strand conformation polymorphism analysis. Mutations of the p53 gene were detected in two of the six SCCs but not in any of the 17 porokeratotic specimens. These two mutations were C to T transitions at codons 146 and 175 in exon 5, which were a nonsense mutation at a dipyrimidine site and a missense mutation at a CG site, respectively. To our knowledge, neither of these mutations has been identified in skin cancers before. Our observations indicate that mutations of the p53 gene are not the major molecular etiology for porokeratosis, but are related to its skin carcinogenesis, and that p53 overexpression in porokeratosis is not due to p53 gene mutations.

Keywords: Porokeratosis; p53 mutation; Squamous cell carcinoma; PCR-SSCP; Immunohistochemistry

1. Introduction

Porokeratosis is a disorder of epidermal keratinization which is histologically characterized by the presence of a column of parakeratotic cells, called the cornoid lamella. Although the disorder is often familial and inherited in an autosomal pattern [1], its pathogenesis is not fully understood yet. Seven variants have been clinically distinguished: the plaque type, giant porokeratosis, disseminated superficial porokeratosis (DSP), disseminated superficial actinic porokeratosis (DSAP), linear porokeratosis, porokeratosis plan-

* Corresponding author.

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Table 1
Summary of the immunohistochemical study and p53 gene mutation analysis

Case no.[a]	Age/sex	Type	Site examined	No. of lesions examined	No. of specimens examined	No. of specimens[b] showing p53 positivity	No. of specimens with p53 gene mutation
1	55/M	Plaque	Forearm	1	1	0	0
2	55/M	Plaque	Lower leg	1	1	1	0
3	26/F	Plaque	Knee	1	1	1	0
4	76/M	Plaque	Upperarm	1	1	1	0
5	60/F	Giant	Hand	1	4	3	0
		SCC	Hand	1	1	1	0
7	76/M	DSP	Abdomen	1	1	1	0
			Forearm	1	1	1	0
8	57/F	DSAP	Forearm	1	1	1	0
9	61/F	DSAP	Lower leg	1	1	1	0
10	55/M	Linear	Trunk	2	2	2	0
			Leg	3	3	2	0
		SCC	Lower leg	5	5	5	2

[a]Number of cases 1 to 9 correspond to those descriped in our previous paper [16]. Case 10 was also reported previously [13].
[b]Immunohistochemical analyses in 19 of 23 specimens used in this study were performed previously [13,16]. Two anti-p53 antibodies, CM1 and DO1, gave the same results in the 23 specimens.

taris palmaris et disseminata, and punctate porokeratosis [1,2]. Occurrence of malignancies in porokeratotic lesions provides clinical evidence for the precancerous nature of this disease [3]. Abnormal DNA ploidy in the porokeratotic epidermis [4] and chromosomal instability of cultured fibroblasts derived from patients with porokeratosis [5,6] may contribute to the premalignant potential of porokeratosis. However, the precise molecular mechanism of the skin carcinogenesis of porokeratosis remains to be elucidated.

Inactivation of the p53 tumor suppressor gene by deletions and/or mutations is a major molecular etiology for human tumorigenesis including skin cancers [7,8]. Immunohistochemical detection of p53 protein has become a common approach for assessing potential p53 mutations in tumors [9], although the correlation between immunohistochemical overexpression of p53 and underlying mutations is not absolute [10,11]. Recent immunohistochemical studies have frequently detected increased p53 expression in the epidermis of porokeratotic lesions [12–16]. We also observed p53 overexpression in squamous cell carcinomas (SCCs) developing on porokeratotic lesions [13,16]. These observations may suggest the involvement of p53 gene mutations in the etiology for porokeratosis itself and/or the subsequent carcinogenesis. Genomic instabilities in porokeratosis [4–6] may also be induced by p53 gene mutations, because cells with p53 gene mutations are genetically less stable [17]. To our knowledge, p53 gene mutations have not yet been studied in porokeratosis or SCC associated with this disease. In this study, we showed frequent p53 overexpression in porokeratotic lesions immunohistochemically. We then screened p53 gene mutations in porokeratotic lesions and SCCs arising on those by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis, and confirmed mutations by DNA sequencing of bands showing mobility shifts.

2. Materials and methods

2.1. Specimens

Table 1 summarizes the details of specimens examined. Briefly, a total of 23 paraffin-embedded specimens were obtained from nine cases of porokeratosis. These included four of the plaque type,

two of DSAP, one of DSP, one of giant porokeratosis with an SCC, and one of linear porokeratosis with multiple SCCs. The 23 specimens consisted of 17 porokeratotic specimens and six SCC specimens. The 17 porokeratotic specimens were obtained from 14 lesions of the nine cases. Of the six SCC specimens, one was obtained from the case of giant porokeratosis and five were obtained from the case of linear porokeratosis. Mutations in the p53 gene were examined in all 23 specimens. However, immunohistochemical p53 expression was studied in four of these 23 specimens, because we had previously examined it in the 19 other specimens (13 porokeratotic and six SCC specimens) [13,16]. Of the four porokeratotic specimens examined immunohistochemically, three were obtained from three different parts of a lesion of giant porokeratosis (case 5 in Table 1). The remaining one was obtained from a patient with DSP (case 7 in Table 1).

2.2. Immunohistochemistry

A rabbit polyclonal anti-p53 antiserum, CM1 (Novocastra Lab., Newcastle upon Tyne, UK), and a mouse monoclonal anti-p53 antibody, DO1 (Oncogene Science, Inc., Manhasset, NY), were used to detect p53 as described previously [13,16]. CM1 and DO1 were applied at a dilution of 1:1000 and a concentration of 1 μg/ml, respectively. In comparison with negative controls using normal rabbit serum for CM1 and normal mouse IgG for DO1, clearly stained nuclei were regarded as specific staining. If a specimen had more than 10 specifically stained nuclei in a selected field magnified 125 times with a Nikon X2F-EFD microscope, it was counted as positive. Occasional staining was considered negative, because it has been found in the normal epidermis [18].

2.3. DNA preparation

DNA was prepared from 10 paraffin-embedded, 6-μm-thick sections on microscopic glass slides as previously described [11,18]. Briefly, the epidermis of porokeratotic lesions was scraped from glass slides with a needle under microscopic observation to enrich epidermal DNA. DNAs of SCC were also prepared from 6-μm-thick sections. Normal tissue DNAs were prepared from either dermal portions of sections or cultured keratinocytes obtained from unaffected skin regions of porokeratosis patients. Leukocyte DNA obtained from a normal subject was also used as a normal control.

2.4. PCR-SSCP analysis and DNA sequencing

Mutations in exons 5 to 10 of the p53 gene were first screened by PCR-SSCP analysis as previously described [11,18]. Oligonucleotide primers used to amplify exons 5 to 10 have been described previously [18]. PCR-SSCP was repeated at least twice in each case showing a mobility shift to ensure that the results were reproducible. Bands with altered migrations in SSCP analysis were extracted from a dried gel. Eluted DNA was re-amplified by PCR and used as a template for DNA sequencing by fluorescence-based dideoxy-termination methods using an Applied Biosystems Model 373A automated DNA sequencer (Perkin Elmer/Applied Biosystems, Foster City, CA) as previously described [11,18].

3. Results

We previously studied immunohistochemical p53 expression in 19 (13 porokeratotic and six SCC specimens) of 23 specimens [13,16]. In this study, we examined this in four other specimens. Of three specimens obtained from a patient with giant porokeratosis, two were immunopositive with CM1 and DO1, and one was immunonegative with both antibodies. One specimen obtained from a patient with DSP was positive with these antibodies. Taken together with previous results [13,16], p53 overexpression was detected in 14 of 17 porokeratotic specimens and all six SCC specimens as summarized in Table 1.

PCR-SSCP analysis of DNAs prepared from the 17 porokeratotic specimens revealed no mobility shift in exons 5 to 10 of the p53 gene. However, two of six SCC DNAs showed mobility shift in exon 5 of the p53 gene (Fig. 1). These two DNAs were those from SCCs developing in a

patient with linear porokeratosis. Their mobility shift patterns were different, indicating the presence of different mutations in these two DNAs.

DNA sequencing of bands showing altered migrations in the two cases revealed point mutations at codons 146 and 175, respectively (Fig. 2). Both mutations were C to T transitions on the transcribed strand. The mutation at codon 146 was a nonsense mutation at a dipyrimidine site. Another mutation at codon 175 was a missense mutation at a CG site, which substituted histidine for arginine. In the previous immunohistochemical study [13], we regarded the SCC with a nonsense mutation as positive because a small cluster of cells stained with CM1 and DO1 were present at the edge of the tumor, although the majority of SCC cells were not stained with these antibodies.

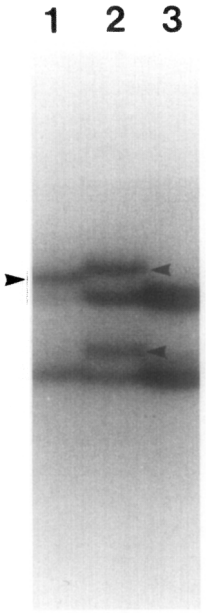

Fig. 1. SSCP analysis of exon 5 of the p53 gene in SCCs arising on porokeratotic lesions. Electrophoresis was performed in a 6% polyacrylamide gel containing 0, 5, or 10% glycerol. The results using a gel containing 5% glycerol are shown. Lane 1, an SCC specimen arising on a porokeratotic lesion on the lower leg of a patient with linear porokeratosis; lane 2, another SCC specimen arising on a different lesion on the lower leg of the same patient; lane 3, normal keratinocyte DNA from the same patient. Two bands showing mobility shifts in lane 2 had an identical sequence after reamplification (Fig. 2). Arrowhead, a band with a mobility shift.

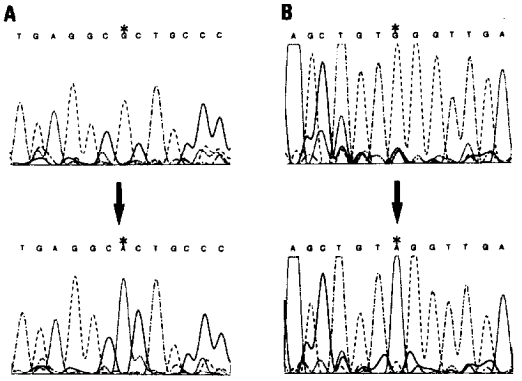

Fig. 2. DNA sequencing of exon 5 of the p53 gene in SCCs arising on porokeratotic lesions. A and B correspond to lanes 1 and 2, respectively in Fig. 1. DNAs which were eluted from bands showing altered migrations in SSCP analysis, were subjected to DNA sequencing after re-amplification by PCR. Sequences of the coding strand are represented. Top, normal sequences of keratinocyte DNA; bottom, mutated sequences of DNAs from the SCCs arising on porokeratotic lesions. Asterisk, the base at which mutation was detected. (A) a missense mutation of a G to A (C to T) transition at codon 175; (B) a nonsense mutation of a G to A (C to T) transition at codon 146.

In another SCC with a missense mutation, the majority of SCC cells were positively stained [13].

4. Discussion

We have shown the overexpression of p53 in 14 of 17 porokeratotic specimens. Other groups also reported frequent p53 overexpression in the epidermis of porokeratotic lesions using immunohistological methods [12,14,15]. Although immunohistological p53 overexpression has often been associated with p53 gene mutations [9], no mutations of the p53 gene were detected in these 17 porokeratotic specimens by PCR-SSCP analysis. In this study, we screened p53 gene mutations in exons 5 to 10. Therefore, there is a possibility that we overlooked mutations outside these exons. However, mutations are infrequent outside exons 5 to 10 [7]. It is also possible that we could not detect clonally expanded mutations in a small area of the porokeratotic epidermis, because the PCR-SSCP analysis used here might not have been sensitive enough to detect such mutations.

Despite these, the absence of p53 gene mutations in porokeratotic lesions indicates that p53 gene mutations are not the major molecular etiology for porokeratosis itself.

The overexpression of p53 is induced not only by p53 gene mutations but also by various types of DNA damaging agents, including ultraviolet (UV) light and ionizing radiation [19]. Recent evidence indicates that various cellular stresses other than DNA damage, such as heat shock, osmotic shock, blockers of the cellular respiratory system, amino acid analogs, hydrogen peroxide, and heavy metals, also induce nuclear accumulation of p53 in human fibroblasts [20]. Based on this information, p53 overexpression may be easily induced in porokeratosis by various cellular stresses, and UV light may be involved in the overexpression in DSAP lesions because they predominantly develop on sun-exposed skin regions. The p53 gene response to DNA damage is regulated by genes other than the p53 gene as shown in ataxia-telangiectasia and Bloom's syndrome [21,22]. Therefore, a genetic abnormality underlying porokeratosis may affect p53 expression in porokeratosis.

Although the involvement of p53 gene mutations has been clearly demonstrated in UV carcinogenesis [23], it has not been studied yet in skin carcinogenesis associated with porokeratosis. In this study, we detected p53 gene mutations in two of the six SCC specimens arising on porokeratotic lesions but in none of the 17 porokeratotic specimens. The frequency of p53 gene mutations in these SCCs is comparable to that in SCCs etiologically related to UV light [8]. These observations suggest that p53 gene mutations are responsible for the progression of porokeratosis to SCC at least in some cases.

Mutations detected here were a nonsense mutation and a missense mutation at codons 146 and 175 in exon 5 of the p53 gene, respectively. To our knowledge, these two mutations have not been identified in skin cancers etiologically related to UV light [8,10,11,23–27], although codon 175 is a hotspot of p53 gene mutations in internal malignancies [7]. The mutation at codon 146 was a C to T transition at a dipyrimidine site. Although UV light predominantly induces this type of mutation [28], it is not specific to UV mutagenesis, and it is induced by reactive oxygen species as well [29]. The mutation at codon 175 was a C to T transition at a CG site. Mutations at this site are probably related to deamination of 5-methylcytosine [30]. Two SCCs carrying these different mutations developed on the lower leg of a patient with linear porokeratosis, which had rarely been exposed to sunlight. UV light may have had a small influence on the p53 gene mutations detected here.

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