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Advances in Brief

p53 Mutations in Basal Cell Carcinomas[1]

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Abstract

Genomic DNA from 14 basal cell carcinoma biopsies was screened for the presence of mutations in the *p53* gene, using the polymerase chain reaction followed by direct DNA sequencing. Heterozygous mutations were detected in 7 of 14 (50%) samples investigated. All mutations were G:C-A:T transitions, and five (71%) of these mutations were transitions at hot spots with CpG sites, three at codon 248 and two at codon 273. The striking similarity of the type of mutations detected in this study and with the UV mutagenesis studies reported in literature suggest the hypothesis that UV may act on the *p53* gene in a carcinogenic-specific fashion.

Introduction

The proliferation of normal cells is thought to be regulated by growth and suppressor signals mediated by protooncogenes and tumor suppressor genes, respectively (1). Alterations in these signaling pathways can allow the cell to progress to neoplastic growth. A large body of data indicates that alterations in the *p53* tumor suppressor gene are important events in the multistep process of carcinogenesis (2). Although the *p53* tumor suppressor gene has been investigated in a variety of human malignancies, to date no information is available on the state of the *p53* gene in BCC.[4]

Basal cell carcinoma is the most common skin cancer and the most common malignancy in humans (3). Its incidence is clearly correlated with exposure to sunlight. The UV portion of the solar spectrum is the most probable cause of this cancer as suggested by evidence that the tumor appears on sun-exposed parts of the body, such as the face and arms. In addition, its incidence is higher in light-skinned individuals (3). Furthermore, genetic disorders such as the basal cell nevus syndrome, an autosomal dominant condition, and xeroderma pigmentosum, an autosomal recessive disorder characterized by hypersensitivity to UV radiation with a reduced capacity for DNA repair, have been associated with the development of BCC. In fact, patients with XP have a > 1000-fold increased frequency of developing skin cancer when exposed to sunlight (4).

In the present study, we screened 14 BCCs for mutations in exons 5 to 9 of the *p53* gene. Mutations were identified by direct sequencing of PCR-amplified genomic DNA.

Materials and Methods

DNA Samples. Basal cell carcinoma biopsies from 14 patients were collected during standard surgical procedure, frozen in liquid nitrogen, and stored in −80°C until DNA extraction. High molecular weight DNA was extracted by the sodium dodecyl sulfate-proteinase K-phenol-chloroform method and precipitated in cold ethanol.

PCR Amplification and Sequencing. Total genomic DNA (0.5 μg) was used in four separate 100-μl PCR assays for 40 cycles (denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min) to generate fragments corresponding to exons 5, 6, 7, 8, and 9. The primer sequences and the corresponding amplified exons are listed in Table 1. All primers were located at exon-intron junctions (5). Amplifications were performed using a Gene Amp PCR Core Reagent kit (Perkin-Elmer-Cetus, Norwalk, CT) according to the instructions of the manufacturer. After amplification, 70 μl of PCR reactions were fractionated in 2% NuSieve GTG (FMC Corp., Rockland, ME) agarose gel electrophoresis. The amplified fragments were cut out and extracted by Qiagen gel extraction kit (Qiagen, Inc., Chatsworth, CA). Dried DNA was resuspended in 25 μl distilled H_2O, and 50 fmol DNA were directly sequenced by the procedure of Sanger *et al.* (6) as modified by Murray (7) using a double-stranded DNA cycle sequencing system (BRL Life Technologies, Gaithersburg, MD) following the manufacturer's instructions. Briefly, the oligonucleotide primers, identical to those used for PCR amplification, were end-labeled with [γ-^{32}P]ATP (ICN Biomedicals, Costa Mesa, CA) in the presence of T4 polynucleotide kinase. The sequencing was performed with Taq DNA polymerase in 25 cycles consisting of two steps of 30 s at 95°C and 20 s at 65°C. The samples were electrophoresed in 8 M urea/6 or 7% Hidrolink Long Ranger gel (AT Biochem, Malvern, PA). The gel was dried and exposed to Hyperfilm β-max X-ray film (Amersham Co., Arlington Heights, IL) at −70°C for 24–48 h.

Results

Genomic DNA from 14 basal cell carcinoma biopsies were examined for alterations in the exons 5 to 9 of the *p53* gene. Sequence analysis of the *p53* gene showed single missense point mutations in 7 samples (Table 2). All point mutations altered the coding sequence and were present in exons 7 and 8. Heterozygous mutations in exon 8 occurred at codon 273 with CGT-TGT resulting in an amino acid change of arginine to cysteine in two tumor samples. The other point mutations were located at exon 7, one at the codon 245 (GGC-AGC, glycine to serine), one at the codon 246 (ATG-ATA, methionine to isoleucine), and three at the codon 248 (two CGG-TGG, arginine to tryptophan; and one CGG-CAG, arginine to glutamine) (Figs. 1 and 2), respectively.

Discussion

Sequence analysis has revealed the presence of five evolutionary conserved domains of *p53*, four of which fall within exons 5–8 (2). The majority of the reported mutations are located within these conserved domains, *i.e.*, in residues 117–142, 171–181, 234–258, and 270–286, with at least three hot spot mutations affecting residues 175, 248, and 273. In this study, we detected single missense mutations of *p53* in 7 of 14 (50%) BCC investigated. All mutations were G:C–A:T transitions and five (71%) of these mutations were transitions at hot spots with CpG sites, three at codon 248 and two at codon 273, respec-

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[4] The abbreviations used are: BCC, basal cell carcinoma; XP, xeroderma pigmentosum; PCR, polymerase chain reaction; SCC, squamous cell carcinoma.

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Table 1 *Oligonucleotide primers*

Primer sequence	Orientation	Amplified *p53* exons
5′-TTCCTCTTCCTGCAGTACTC-3′	Sense	5 and 6
5′-AGTTGCAAACCAGACCTGAG-3′	Antisense	5 and 6
5′-GTGTTR[a]TCTCCTAGGTTGGC-3′	Sense	7
5′-CAAGTGGCTCCTGACCTGGA-3′	Antisense	7
5′-CCTATCCTGAGTAGTGGTAA-3′	Sense	8
5′-TCCTGCTTGCTTACCTCGCT-3′	Antisense	8
5′-AAGCGAGGTAAGCAAGCAGG-3′	Sense	9
5′-CCCAAGACTTAGTACCTGAA-3′	Antisense	9

[a] R, A/G.

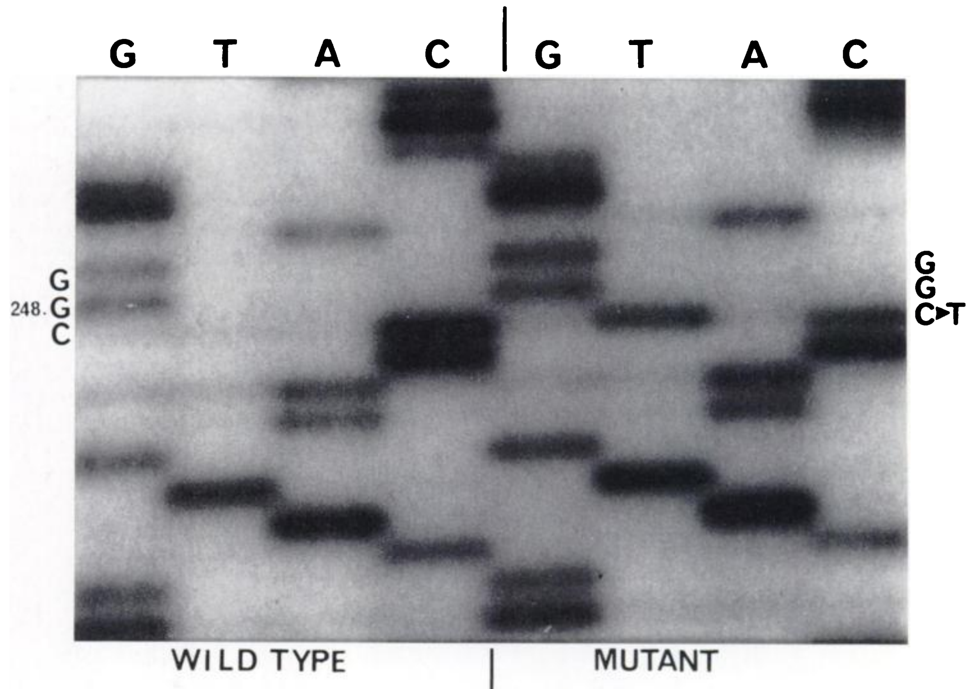

Fig. 1. DNA sequence autoradiograph of exon 7 of the *p53* gene in the region of codon 248. A heterozygous C–T transition was detected, resulting in the substitution of arginine for tryptophan.

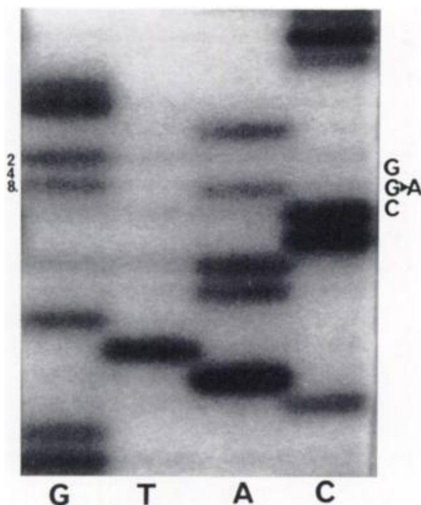

Fig. 2. DNA sequence autoradiograph of exon 7 of the *p53* gene by direct sequencing of the PCR product. A heterozygous G–A transition was detected in the codon 248, resulting in the substitution of arginine for glutamine.

tively. Transitions at CpG dinucleotides are frequent, representing about one-third of all the *p53* mutations reported in various neoplasias, and the frequency of these transitions varies with the type of cancer (2).

Sunlight-induced human skin cancers are most likely caused by DNA damage produced by the UV portion of the solar spectrum. The current paradigms for the molecular steps in carcinogenesis, activation of protooncogenes, or inactivation of tumor suppressor genes suggest these genes as targets for the mutagenic effects of UV. Our results may indicate that the UV acts on the *p53* gene in a carcinogen-specific fashion. This hypothesis is suggested by the observation that the point mutations detected in BCC are strikingly similar to the results of UV mutagenesis studies in human cells. These studies have demonstrated that the UV mutations were predominantly C–T transitions (8–10). In the *Escherichia coli supF* gene treated with UV and introduced in human cells from xeroderma pigmentosum, a DNA repair-deficient disease, and in human repair-proficient cells (9), two notable differences emerged between XP and normal cells: (*a*) 93% of the mutant plasmids from the XP cells showed G:C–A:T transitions, whereas they represented only 73% in the normal cells; and (*b*) the reduction of percentage of transversions from 25% in the normal cells to 4% in the XP cells, suggesting that either transversion events originate as errors in excision repair, or that the transversion photoproduct is so lethal when unrepaired that mutations due to this photoproduct are underrepresented in the mutation spectrum. Furthermore, in a recent study on the state of the *p53* gene in SCC of the skin (11), a malignancy clearly related to the action of UV, the investigators reported the presence of *p53* mutations in 58% of SCC samples with a large predomi-

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Table 2 *Heterozygous mutations in p53 gene in human basal cell carcinoma*

Tumor	Exon	Codon	Mutation[a]	Amino acid substitution
1	7	245	GGC-AGC	Gly-Ser
2	7	246	ATG-ATA	Met-Ile
3	7	248	CGG-TGG	Arg-Trp
4	7	248	CGG-TGG	Arg-Trp
5	7	248	CGG-CAG	Arg-Gln
6	8	273	CGT-TGT	Arg-Cys
7	8	273	CGT-TGT	Arg-Cys

[a] Boldface print, bases mutated.

nance of mutations of the transition type. These investigators examined exons 2 and 4–9, while only exons 5–9 were investigated in the present study. Furthermore, Brash *et al.*, discovered that 3 of 24 SCC contained CC-TT double base changes, which are thought to be specific to UV. None of the BCC in the present study, however, were found to contain these double base changes. These data, as well as our study on BCC, are suggestive of the *p53* gene as one of the predominant target genes of UV light in UV-related malignancy.

References

1. Weinberg, R. A. Tumor suppressor genes. Science (Washington DC), *254:* 1138–1146, 1991.
2. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. *p53* mutations in human cancers. Science (Washington DC), *253:* 49–53, 1991.
3. Miller, S. J. Biology of basal cell carcinoma (Part 1). J. Am. Acad. Dermatol., *24:* 1–13, 1991.
4. Kraemer, K. H., Lee, M., and Scotto, J. DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. Carcinogenesis (Lond.), *5:* 511–514, 1984.
5. Buchman, V. L., Chumakov, P. M., Ninkina, N. N., Samarina, O. P., and Georgiev, G. O. A variation in the structure of the protein-coding region of the human *p53* gene. Gene, *70:* 245–252, 1988.
6. Sanger, F., Nicklen, S., and Coulson, A. R. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA, *74:* 5463–5467, 1977.
7. Murray, V. Improved double-stranded DNA sequencing using the linear polymerase chain reaction. Nucleic Acids Res., *17:* 8889, 1989.
8. Glazer, P. M., Sarkar, S. N., and Summers, W. C. Detection and analysis of UV-induced mutations in mammalian cell DNA using a λ phage shuttle vector. Proc. Natl. Acad. Sci. USA, *83:* 1041–1044, 1986.
9. Bredberg, A., Kraemer, K. H., and Seidman, M. M. Restricted ultraviolet mutational spectrum in a shuttle vector propagated in xeroderma pigmentosum cells. Proc. Natl. Acad. Sci. USA, *83:* 8273–8277, 1986.
10. Seidman, M. M., Bredberg, A., Seetharam, S., and Kraemer, K. H. Multiple point mutations in a shuttle vector propagated in human cells: evidence for an error-prone DNA polymerase activity. Proc. Natl. Acad. Sci. USA, *84:* 4944–4948, 1987.
11. Brash, D. E., Rudolph, J. A., Simon, J. A., Lin, A., McKenna, G. J., Baden, H. P., Halperin, A. J., and Pointen, J. A role for sunlight in skin cancer: UV-induced *p53* mutations in squamous cell carcinoma. Proc. Natl. Acad. Sci. USA, *88:* 10124–10128, 1991.

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