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Progression of Basal Cell Carcinoma through Loss of Chromosome 9q and Inactivation of a Single *p53* Allele

Peter van der Riet, Debra Karp, Evan Farmer, Qingyi Wei, Lawrence Grossman, Kaori Tokino, J. Michael Ruppert, and David Sidransky[1]

Head and Neck Cancer Research Division, Department of Otolaryngology [P. v. d. R., K. T., J. M. R., D. S.], and Departments of Dermatology [D. K., E. F.] and Biochemistry [Q. W., L. G.], School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205

Abstract

Basal cell carcinoma (BCC) of the skin represents a unique group of tumors strongly associated with exposure to UV light. Unlike squamous carcinoma of the skin, BCC is generally indolent, noninvasive, and rarely metastatic. To study the involvement of tumor suppressor genes in these neoplasms, we analyzed 36 BCCs for *p53* mutations and a subset of these tumors for loss of chromosomes 17p and 9q. Sixty-nine % of sporadic BCCs had lost a 9q allele, with the common area of loss surrounding the putative gene for nevoid BCC or Gorlin's syndrome. Forty-four % (16 of 36) of BCCs had a mutated *p53* allele, usually opposite pyrimidine tracts, which is consistent with UV-induced mutations. Surprisingly, only one tumor had lost a 17p allele, and in all BCCs only one *p53* allele was inactivated. This is in direct contrast to other epithelial tumors, which usually progress by the inactivation of both *p53* alleles.

Introduction

Basal cell carcinoma is the most common malignancy in human beings, and the incidence continues to increase (1). In the United States alone over 500,000 basal cell carcinomas occur annually, usually in the elderly light-skinned population and after prolonged exposure to sunlight. These tumors are composed of rather undifferentiated basal keratinocytes, generally grow slowly, and behave in a relatively benign and nonaggressive fashion. However, substantial morbidity, including local tissue destruction and disfigurement, is associated with these tumors (2).

Cancers are now known to arise through a series of well-defined genetic changes that accumulate during histopathological progression (3). The most common of these genetic changes in human cancers are alterations of the *p53* tumor suppressor gene (4). In colorectal cancer, alterations of *p53* occur in the transition from the benign (adenomatous) to the malignant (carcinomatous) state (3, 5). Inactivation of *p53* occurs predominantly by point mutation of one allele followed by loss of the remaining wild type allele, as hypothesized by Knudson (6). These events result in loss of heterozygosity for polymorphisms in the chromosomal region surrounding the *p53* gene.

Although basal cell carcinomas are very common, very little is known about their molecular pathogenesis (2). Recent studies have reported a predominance of UV-induced *p53* mutations in BCC[2] (7–9). The persistence of UV-induced photoproducts in DNA has been attributed to reduced levels of DNA repair in BCC (10). Additionally, loss of heterozygosity of chromosome 9q has been found in sporadic and hereditary BCC (11). To further define the involvement of tumor suppressor genes in the molecular pathogenesis of epithelial skin cancers, we screened basal cell carcinomas for *p53* gene mutations and allelic loss of chromosomes 17p and 9q. Although these tumors have a high percentage of *p53* alterations, the basal cell carcinomas we studied appeared to harbor only a single inactivated *p53* allele.

Materials and Methods

Archived paraffin blocks of basal cell cancer biopsies from patients at The Johns Hopkins Hospital Dermatopathology Laboratory were collected. A hematoxylin/eosin-stained formalin-fixed biopsy section was viewed under the microscope for each tumor, and only the corresponding tumor tissue of an unstained 4-μm paraffin section was scraped away from glass slides and then deparaffinized in xylene. The tissue was then digested with sodium dodecyl sulfate/proteinase K and DNA was extracted with phenol/chloroform followed by ethanol precipitation. Stored lymphocytes from these BCC patients were used to isolate normal DNA for comparison with their tumors to determine whether allelic loss had occurred.

The *p53* gene was amplified in two segments. One segment included exons 5 and 6 utilizing primers 5′-CAU CAU CAU CAU TTC ACT TGT GCC CTG ACT T-3′ and 5′-CUA CUA CUA CUA CCA CTG ACA ACC ACC CTT-3′, and the other segment included exons 7 and 8 utilizing primers 5′-CAU CAU CAU CAU CCA AGG CGC ACT GGC CTC-3′ and 5′-CUA CUA CUA CUA CTG GAA ACT TTC CAC TTG AT-3′. Following amplification with the uracil-containing primers, the PCR products were extracted with phenol/chloroform and run on a 1% agarose gel. The DNA product was cut from the gel, extracted, and treated with uracil-DNA glycosolase. One-half of the total product was annealed to the Cloneamp (BRL) plasmid vector according to the manufacturer's instructions (12). Competent DH5-α cells were transfected with plasmid by heat shock, plated on ampicillin plates, and incubated overnight at 37°C. More than 100 colonies were pooled and plasmid DNA was isolated by alkaline lysis followed by isopropyl alcohol precipitation.

Double-stranded DNA obtained from plasmid was sequenced by the dideoxy method utilizing Sequenase (USB) enzyme and [^{33}P]dATP as described (13). Prior to termination, a 30-min incubation at 37°C with 0.5 unit of Klenow enzyme (NEB) helped to eliminate "stop" bands. Sequencing reactions products were then separated on a 6% urea/polyacrylamide gel and exposed to film.

Microsatellite markers *D9S145, D9S146, D9S12* (1.1, 1.2), *D9S12* (2.1, 2.2), *D9S53*, and *D9S123* on chromosome 9q and markers *CHRNB1, D17S122*, and *D17S786* on chromosome 17p were used in PCR analysis (Research Genetics, Huntsville, AL) to detect dinucleotide repeat polymorphisms in normal and tumor tissue from patients with basal cell carcinoma. For PCR amplification of polymorphic repeats, 50 ng of each primer were end labeled with [γ-^{32}P]ATP (20 μCi; Amersham) and T_4 kinase (NEB) in a total volume of 50 μl. PCR reactions were carried out in a total volume of 25 μl containing 50 ng of genomic DNA, 2.5 ng of labeled primer, 50 ng of each unlabeled primer, 16.6 mM NH_4SO_4, 67 mM Tris (pH 8.8), 6.7 mM $MgCl_2$, 10 mM β-mercaptoethanol, 6.7 μM EDTA, 1% dimethyl sulfoxide, 1.5 mM deoxyribonucleotide triphosphatess, and 5 units of AmpliTaq DNA polymerase. The amplification for each primer set was performed at 95°C for 30 s, 50–58°C for 30 s, and 70°C for 30 s, a total of 25 to 30 cycles. One-tenth of the PCR products were separated on a 6% urea-polyacrylamide gel and exposed to film.

Results

***p53* Mutations in Basal Cell Carcinomas.** We analyzed 36 basal cell carcinomas by sequencing exons 5–8 of the *p53* gene. Forty-four % (16 of 36) of the basal cell carcinomas were found to contain *p53*

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[1] To whom requests for reprints should be addressed.

[2] The abbreviations used are: BCC, basal cell carcinomas; PCR, polymerase chain reaction.

mutations (Table 1). Closer analysis of the specific mutations in BCCs revealed that 69% were missense mutations, 6% were nonsense mutations, and 25% were frame shifts. Two-thirds (8 of 12) of the point mutations occurred at or adjacent to dipyrimidines. In all the mutated samples, the sequencing autoradiograph revealed both a wild type and a mutated allele. In addition, the reduction of the wild type sequence signal was always less than 50% in these cases. This may represent some residual contamination from surrounding normal tissue or it may be that the second *p53* allele was retained in these lesions. To further assess the status of the second *p53* allele, we looked at polymorphic markers on chromosomes 17p and 9q.

Allelic Loss of Chromosomes 9q and 17p. We obtained matched lymphocytes and tumor DNA from 18 of the 36 basal cell carcinoma patients. These patients were selected simply on the basis of availability of normal DNA for this study. Paired samples of normal and tumor DNA were amplified by PCR for analysis of polymorphic dinucleotide repeats. Allelic loss was scored if either of the two alleles present in the normal cells was absent in the DNA from the tumor cells.

Chromosome 17p was tested with three markers and exhibited allelic loss in only 1 (8%) of the 16 tumors. No tumor with a *p53* mutation was found to have allelic loss on 17p. Additionally, the one tumor with 17p loss did not have a *p53* mutation, confirming the initial observations from the sequencing data.

We used 6 markers to test allelic loss on chromosome 9q and found loss of heterozygosity in 69% (11 of 16) of the informative tumors. The common deleted area included *D9S12*, a marker closely linked with an inherited predisposition for basal cell carcinoma (Gorlin's syndrome) (11). There was a close association of 9q loss with *p53* alterations, inasmuch as 8 of 11 tumors with 9q loss also had a *p53* alteration. If 9q loss generally occurs first, a *p53* mutation apparently occurs rapidly in BCC progression. Fig. 1 shows tumor 35 with loss of chromosome 9q and a *p53* mutation with retention of the wild type band on the sequencing gel. Because 11 of the tumors demonstrated complete loss of 9q, they clearly illustrate the overall purity of the tumor tissue and the definitive retention of one *p53* allele during progression.

As a further control, we sequenced exons 5–8 of the *p53* gene in 14 squamous cell carcinomas of the skin and found mutations in 3 of them, with complete loss of the wild type allele in 2 of 3 (data not shown), suggesting that squamous carcinomas of the skin progress by the usual inactivation of both *p53* alleles. Similar results for squamous carcinomas have been described elsewhere (14).

Discussion

The genetic analysis of basal cell carcinoma from paraffin-embedded tissue is very tedious because the carcinomas are often very small at the time of diagnosis. Therefore, genetic alterations in tumor cell DNA can be masked by the presence of DNA from admixed cells. To ensure that we had pure neoplastic tissue we carefully microdissected each lesion. Our results are in agreement with the finding of *p53* mutations in 48–50% of basal cell carcinomas by others (7, 8). These investigators also noted that the wild type *p53* allele appeared to be retained in all tumors, but the possibility of contaminating nontumorous cells could not be excluded. Our results are also in agreement with Ziegler *et al.* (9), who found that 56% of their basal cell carcinomas of the skin contained *p53* mutations. However, some of their tumors (4 of 15) had either a second *p53* mutation on the remaining allele or, in one case, loss of heterozygosity on 17p. It is possible that we

Table 1 *P53 Mutations and chromosomal loss in BCC*

Tumor	Age (yr)	Sex	Pyrimidine tract	Base pairchange	Amino acid change	Codon	Chromosome	
							17p	9q
1	72	M	CC→CT	ggCCCat→ggCTGat	Pro→Leu	250	—[a]	—
2	62	M	—	ND	—	—	—	—
3	82	F	—	gAGCa→gACCa	Ser→Thr	303	—	—
4	55	M	CC→TC	tGGGa→tAGGa	Gly→Arg	279	—	—
5	74	M	—	ND	—	—	—	—
6	63	M	—	ND	—	—	—	—
7	58	M	—	ND	—	—	—	—
8	64	F	—	ND	—	—	—	—
9	58	M	—	ND	—	—	—	—
10	55	F	—	ND	—	—	—	—
11	70	M	—	ND	—	—	—	—
12	59	F	CC→TC	ggcGGC→ggcAGC	Gly→Ser	245	—	—
13	57	M	—	ND	—	—	—	—
14	58	M	—	ND	—	—	—	—
15	57	M	CC→TT	cCCCca→cCTTca	Pro→Leu	177	—	—
16	56	M	—	tgCGT→tgCCT	Arg→Pro	202	—	—
17	57	M	CC→TT	ttATCCGAgt→ttATTTGAgt	Ile-Arg→Ile-stop	196	—	—
18	56	M	—	ND	—	—	—	—
19	58	M	—	CCGGCA→CCGGGCA	Frame shift	154	NLH[b]	LOH
20	56	M	—	ttatct→ttaact	Frame shift	Intron 6	NI	LOH
21	57	M	—	gCGTg→gCATg	Arg→His	273	NI	LOH
22	59	F	—	CCTGGG→CCTTGGG	Frame shift	278	NI	NLH
23	58	M	—	ND	—	—	LOH	LOH
24	57	M	—	ND	—	—	NLH	LOH
25	46	M	TT→TC	gAACt→cAGCt	Asn→Ser	235	NLH	LOH
26	46	M	—	ND	—	—	NLH	LOH
27	46	M	—	ND	—	—	NLH	NLH
28	37	M	—	ND	—	—	NLH	NI
29	49	M	ccA→ccT	ccAGGt→ccTGGt	Arg→Trp	261	NLH	LOH
30	58	F	—	ND	—	—	NLH	NLH
31	50	M	ACc→GCc	aCTGg→aCCGg	Leu→Pro	257	—	LOH
32	43	F	—	ND	—	—	NI	NLH
33	38	F	—	ND	—	—	NLH	NLH
34	46	M	—	ND	—	—	NLH	LOH
35	45	M	—	gCGTg→gCATg	Arg→His	273	NLH	LOH
36	36	M	—	caagggt→caaggt	Frame shift	intron 8	NLH	NLH

[a] A dash (—) indicates insufficient DNA for analysis.
[b] NLH, no loss of heterozygosity; LOH, loss of heterozygosity; NI, noninformative; ND, no *p53* mutation detected.

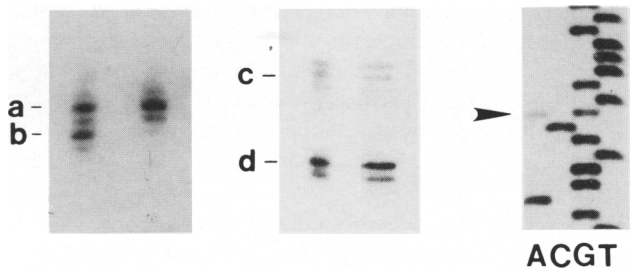

Fig. 1. Allelic loss and *p53* mutations in BCC. Tumor 35 (alleles *a* and *b*) reveals complete loss of allele *b* on chromosome 9q and retention of both alleles on chromosome 17p (*c* and *d*) using microsatellite markers. A sequencing gel autoradiograph demonstrates the presence of a G to A change in codon 273 (*arrowhead*) in tumor DNA with retention of the wild type *p53* band. Methods for amplification of microsatellite markers and *p53* sequencing are described in the text.

missed a small number of mutations that are outside the conserved regions that we sequenced. However, the bulk of the data from all the studies combined suggests that basal cell carcinomas commonly progress through inactivation of a single *p53* allele.

Mutation of *p53* may produce a possible dominant protooncogene able to add malignant potential to tumors (15, 16). This so-called "gain of function" *p53* mutation could be the predominant mechanism of progression for basal cell carcinoma. However, the fact that some mutations result in truncations and have not been previously described as "gain of function" mutants would argue against this. Alternatively, the well-established tumor-suppressing function of *p53* could be diminished by inactivation of a single allele (17, 18). The fact that these tumors do not commonly contain an inactivated second *p53* allele remains an interesting phenomenon. Unlike other epithelial tumors, which can progress and invade, these tumors appear to behave in a more benign fashion (1, 2), further suggesting a different genotype from that of invasive squamous carcinomas of the skin. Perhaps basal cell carcinomas that do invade and act aggressively may somehow inactivate both *p53* alleles (19, 20).

It is also possible that *p53* in these cells is an essential protein (as opposed to normal cells) and as a single transformed cell loses the second *p53* allele, it cannot proceed to clonal outgrowth. Given the role of *p53* in G_1/S arrest after DNA damage (21, 22), it can be hypothesized that BCCs have undergone a genetic change that requires the p53 cell cycle checkpoint for viability. This might be analogous to certain mutations in yeast that render the cells nonviable in the absence of checkpoint functions such as MEC1 (mitosis entry checkpoint) (23).

The spectrum of *p53* mutations is remarkably similar among our tumors and those of previous studies. A preponderance of mutations among pyrimidine tracts has now been described for a variety of skin cancers, presumably caused by exposure to UV light (7, 8, 14). The specific dinucleotide base pair changes secondary to UV-induced pyrimidine dimers are rarely seen in visceral tumors, except those that may be endogenously induced by superoxides derived from inflammatory cells (24, 25).

The majority of our BCCs exhibited a clear loss of chromosome 9q, which agrees with the results of one previous study (11). A minimal area of loss is localized to the putative tumor suppressor gene responsible for nevoid BCC (Gorlin's syndrome), a disease with developmental anomalies and a significant predisposition to develop multiple basal cell carcinomas of the skin (12). It is tempting to speculate that loss of 9q may be an early genetic change that is followed by inactivation of a single *p53* allele. In colorectal cancer, the inactivation of the *APC* gene on 5q is responsible for hereditary familial adenomatous polyposis (26) and also for the initiation of a majority of sporadic tumors (27). However, in BCC, such a close association exists between loss of 9q and a *p53* alteration that we cannot statistically distinguish which event is likely to occur first. Isolation of the gene responsible for Gorlin's syndrome and initiation of sporadic basal cell carcinomas might lead to further understanding of these common cancers. Finally, the function of *p53* in BCC cells may shed light on the normal, complex physiology of the p53 protein and its role in cancer progression.

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