p53 Mutations and Chromosome Instability in Basal Cell Carcinomas Developed at an Early or Late Age

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ABSTRACT

Tumor DNA from 45 primary basal cell carcinoma (BCC) biopsies was screened for p53 gene mutations, chromosome 9 allele loss, and microsatellite instability. p53 mutation frequency increased significantly as a function of the age at BCC onset ranging from 6% (1/16) in early BCC (before age 40 years) to 35% (10/29) in late BCC. All p53 mutations found implicated sunlight as the mutagen. Chromosome 9 instability (allele loss or microsatellite instability) was detected at high frequency (38%) independently of age at tumor onset. Allelic loss was confined to chromosome 9q, whereas microsatellite instability was observed prevalently on chromosome 9p often in association with a replication error (RER+) phenotype. Most of our late BCC patients reported occupational sun exposure, while early BCC patients recalled childhood (0–20 years) recreational sun exposure. These data suggest that chronic exposure to sunlight is responsible for accumulation of p53 mutations and thus for late BCC appearance, whereas acute UV exposure in childhood and adolescence leads to early skin cancer development in genetically susceptible individuals via a p53-independent pathway.

INTRODUCTION

NMSC, including BCC and squamous cell carcinoma, is the most common neoplasia among the white population. Sunlight exposure is accepted to be the most important environmental risk factor while ethnic origin and pigmentedary characteristics are considered the genetic risk factors for this disease. Large case-control studies have been conducted to evaluate the role played by environmental versus genetic factors in skin cancer risk (reviewed in Ref. 1). Little information is available on the populations of southern countries which, as compared to the north, present a lower although relatively high incidence of skin cancer. In these populations, skin cancer risk factors other than pigmentedary characteristics might exist.

Data from Italian cancer registries (2) indicate an incidence of NMSC of 50/100,000/year for males and 37/100,000/year for females which is intermediate when compared with worldwide figures. This rate should be regarded as an underestimate, given the inevitable high proportion of cases which escape registration. Although the reported frequency of distant metastases for NMSC is estimated to be only 0.1% of primary tumors, these are locally destructive and have a tendency to recur. These features make this type of cancer an important public health issue.

Among NMSC, BCC is the most common skin cancer, usually associated with old age. The accumulation of UV-induced mutations in critical genes for skin cancer development is a plausible etiological model for late BCC onset. In fact, a high prevalence (12–58%) of p53 mutations has been reported in BCC (3–8); the mutations observed model for late BCC onset. In fact, a high prevalence (12–58%) of p53 instability (allele loss or microsatellite instability) was detected at high frequency (38%) independently of age at tumor onset. Allelic loss was confined to chromosome 9q, whereas microsatellite instability was observed prevalently on chromosome 9p often in association with a replication error (RER+). Most of our late BCC patients reported occupational sun exposure, while early BCC patients recalled childhood (0–20 years) recreational sun exposure. These data suggest that chronic exposure to sunlight is responsible for accumulation of p53 mutations and thus for late BCC appearance, whereas acute UV exposure in childhood and adolescence leads to early skin cancer development in genetically susceptible individuals via a p53-independent pathway.

MATERIALS AND METHODS

BCC Cases. BCC patients were enrolled at the Istituto Dermopatico dell’Immacolata, a large hospital for skin diseases in Rome which serves as a major source of both outpatient and inpatient dermatological care for central and southern Italy. A total of 45 patients diagnosed with primary BCC were randomly selected from the population of an ongoing case-control study of risk factors for BCC. Each patient was interviewed at the hospital by a trained interviewer using a standardized questionnaire which included demographic data and information on pigmentedary and constitutional characteristics and history of sunlight exposure.

p53 Mutation Analysis. BCC paraffin-embedded biopsies were obtained from each patient. A stained biopsy section was viewed under the microscope and only the corresponding tumor tissue from an unstained paraffin section was microdissected. DNA was extracted by incubating individual 10-μm histological sections in 0.5 ml of buffer containing 10 mm Tris pH 8.3, 50 mm KCl, 1.5 mM MgCl2, 100 μg/ml BSA, 1% Tween 20, and 100 μg/ml proteinase K (15). The sections were incubated overnight at 55°C, boiled for 5 min, and then cooled on ice. Aliquots of 5 μl were used in PCRs. Exons 5–8 of the p53 gene were amplified by 30 PCR cycles at 95°C for 2 min, 55°C for 2 min, and 72°C for 3 min. The amplimers used for p53 gene amplification were: exon 5, 5'-TGT TCA CTT GTG CCA CTG-3' (sense) and 5'-CAG CCC TGT CGT CTG TCC AG-3' (antisense); exon 6, 5'-GGC CTC TGA TFC CTC and only the corresponding tumor tissue from an unstained paraffin section

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1 To whom requests for reprints should be addressed.
2 The abbreviations used are: NMSC, nonmelanoma skin cancer; BCC, basal cell carcinoma; LOH, loss of heterozygosity; NBCCS, nevoid basal cell carcinoma syndrome; MI, microsatellite instability; RER, replication error; AK, actinic keratosis.

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(sense) and 5'-TGT GCA GGG TGG CAA GTG C-3' (antisense); and exon 8, 5'TGC TTC TCT TTT CCT ATC CTG A3' (sense) and 5'CGC TTC TGG TCC TGG CT3' (antisense). The antisense primer was biotinylated at the 5' end to allow the purification of single-stranded DNA for sequencing analysis. The sequencing products were separated on a 6% urea/polyacrylamide gel and exposed to film.

**Analysis of LOH and MI.** Matched lymphocyte and tumor DNA was obtained from 42 of 45 BCC patients analyzed for p53 mutations. Genomic DNA was extracted from both isolated lymphocytes and BCC biopsies of the same patient. DNA was prepared from lymphocytes by using standard methods of phenol/chloroform extractions followed by ethanol precipitation. Four microsatellite probes were selected for chromosome 9 analysis: D9S157, D9S171, D9S169 (9p), and D9S180 (9q). When LOH or MI were detected on chromosome 9, the analysis was extended to three other loci on different chromosomes by using the following microsatellite probes: D2S119, D2S123, and D10S186. The sequences of all microsatellite probes have been reported previously (16).

Some 9, the analysis was extended to three other loci on different chromosomes by using the following microsatellite probes: D2S119, D2S123, and D10S186. The sequencing products were separated on a 6% urea/polyacrylamide gel and transferred to Hybond N+ membranes (Amersham Italia, Milan, Italy). One of the PCR primer oligonucleotides radiolabeled with α-32P by terminal deoxynucleotidyltransferase (Life Technologies Italia, Milan, Italy) was used as hybridization probe to detect the amplification products. Hybridization was performed overnight at 42°C in 130 mM sodium phosphate (pH 7.0), 250 mM NaCl, 10% polyethylene glycol (M, 4000; Sigma Chemical Co., St. Louis, MO) and 7% SDS. Hybridization products were detected by autoradiography. MI was scored as an altered band pattern. A tumor was defined as having a RER (RER+) phenotype when instability was observed at more than three loci on different chromosomes. LOH was defined as a reduction of at least 50% of the signal of one allele as compared with the corresponding normal allele.

**RESULTS**

**Pigmentary Features and Sun-related Behavior of BCC Cases.** Table 1 shows the variables reflecting pigmentary and constitutional traits and sun exposure behavior of the 45 BCC patients of this study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall</th>
<th>21—40 yr</th>
<th>41—60 yr</th>
<th>61—80 yr</th>
<th>χ2 test (P value)</th>
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<tr>
<td><strong>Sex</strong></td>
<td>90</td>
<td>62</td>
<td>24</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25 (55.5)</td>
<td>7 (43.7)</td>
<td>8 (51.7)</td>
<td>10 (66.7)</td>
<td>0.434</td>
</tr>
<tr>
<td>Female</td>
<td>20 (44.5)</td>
<td>5 (36.5)</td>
<td>6 (42.9)</td>
<td>3 (18.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Tumor site</strong></td>
<td>34 (75.5)</td>
<td>11 (68.7)</td>
<td>12 (80.0)</td>
<td>11 (68.7)</td>
<td>0.298</td>
</tr>
<tr>
<td>Sun-exposed</td>
<td>5 (11.1)</td>
<td>1 (6.3)</td>
<td>4 (28.6)</td>
<td>1 (6.3)</td>
<td></td>
</tr>
<tr>
<td>Non-sun-exposed</td>
<td>29 (64.4)</td>
<td>6 (37.5)</td>
<td>10 (71.4)</td>
<td>5 (33.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Eye color</strong></td>
<td>16 (35.6)</td>
<td>9 (56.3)</td>
<td>4 (28.6)</td>
<td>5 (33.3)</td>
<td>0.671</td>
</tr>
<tr>
<td>Blue/green/gray</td>
<td>34 (75.5)</td>
<td>12 (75.0)</td>
<td>10 (66.7)</td>
<td>8 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 (11.1)</td>
<td>3 (18.7)</td>
<td>0 (0)</td>
<td>2 (13.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Color</strong></td>
<td>40 (88.9)</td>
<td>22 (68.7)</td>
<td>14 (71.4)</td>
<td>8 (66.7)</td>
<td>0.250</td>
</tr>
<tr>
<td><strong>Skin type</strong></td>
<td>25 (55.5)</td>
<td>10 (66.7)</td>
<td>9 (50.0)</td>
<td>6 (42.9)</td>
<td>0.483</td>
</tr>
<tr>
<td>Type II</td>
<td>20 (44.4)</td>
<td>12 (75.0)</td>
<td>9 (50.0)</td>
<td>4 (28.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Type III—IV</td>
<td>5 (11.1)</td>
<td>2 (12.5)</td>
<td>1 (6.3)</td>
<td>1 (6.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Outdoor job (yr)</strong></td>
<td>28 (62.2)</td>
<td>17 (50.0)</td>
<td>10 (33.3)</td>
<td>1 (6.3)</td>
<td></td>
</tr>
<tr>
<td>≤5</td>
<td>17 (37.8)</td>
<td>12 (50.0)</td>
<td>9 (66.7)</td>
<td>1 (6.3)</td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>11 (24.5)</td>
<td>6 (37.5)</td>
<td>1 (6.3)</td>
<td>1 (6.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Recreational sun exposure</strong></td>
<td>34 (75.5)</td>
<td>12 (75.0)</td>
<td>10 (66.7)</td>
<td>8 (50.0)</td>
<td></td>
</tr>
<tr>
<td>before age 20 (wk/yr)</td>
<td>28 (62.2)</td>
<td>12 (75.0)</td>
<td>4 (28.6)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>≤4</td>
<td>17 (37.8)</td>
<td>10 (50.0)</td>
<td>4 (28.6)</td>
<td>4 (28.6)</td>
<td></td>
</tr>
<tr>
<td>&gt;4</td>
<td>33 (73.3)</td>
<td>20 (50.0)</td>
<td>15 (100)</td>
<td>0 (0)</td>
<td>0.007*</td>
</tr>
</tbody>
</table>

* Fisher’s exact test.

These data are displayed by age groups. The majority (43/45) of these subjects resided in central Italy and were almost equally distributed among three age groups: 21—40 years, 16 subjects; 41—60 years, 14 subjects; and 61—80 years, 15 subjects. Males and females were also equally represented. Subjects with the features usually associated with an increased risk of BCC (e.g., light hair, eye, and skin color) were evenly distributed among young and old BCC patients, with a slightly larger number in the youngest age group. Preliminary data from the ongoing case-control study (including 136 cases and 124 controls) indicate a lack of association between pigmentary and constitutional characteristics and risk of BCC in our population (data not shown), thus supporting the hypothesis that the phenotypic traits are a poor predictor of BCC risk.

Interestingly, the history of sun exposure highlighted different sun-related behaviors of the subjects as a function of their age at BCC onset. A significantly higher number of BCC cases with a long history of occupational sun exposure (>5 years) was observed in the oldest age group (χ2 test, one-tailed, P = 0.008). Conversely, the prevalence of subjects with acute recreational sun exposure (>4 weeks), particularly in childhood and adolescence, was significantly higher in the youngest age group (χ2 test, one-tailed, P = 0.0003). The preliminary analysis of our case-control study shows the same age-related pattern of UV exposure for BCC cases but not for the controls (data not shown), further supporting a role for sun exposure behavior in the risk of early versus late BCC onset.

**p53 Mutation Frequency and Type.** Paraffin sections of BCC were surveyed for p53 gene mutations. The majority of the tumors analyzed were from sun-exposed areas, mainly the head (75%), and the remaining 25% (10/45) were localized on body sites only occasionally sun-exposed such as trunk, upper limbs, and legs. The noduloculcerative tumor type was the most common clinicopathological form of BCC among our samples, whereas the superficial type represented only 10% of the BCC analyzed. All tumors were first occurrence BCC.

DNA sequencing of the conserved region (exons 5—8) of the p53 gene revealed that the frequency of p53 mutations changed significantly as a function of the age at BCC onset. As shown in Fig. 1, only one p53 mutation was detected among the 16 tumor biopsies (6%) from the age group 21 to 40 years, whereas a p53 mutation frequency of approximately 35% (10/29) was observed in the age group from 41 to 80 years (Fisher’s exact test, one-tailed, P = 0.035). The type of
p53 mutations detected were exclusively base substitutions located at dipyrimidine sequences and the majority were C → T transitions (46%; Table 2). Two mutations (tumors 6299 and 10518) were found at adjacent pyrimidines (15%) and two tumors (1705 and 4102) harbored double non tandem mutations. All mutations were at template C and most were located at the 3’C in 5’(TC)C3’ or at the central C of short pyrimidine runs. This mutational spectrum is expected to occur if UV photoproducts are fixed into mutations. The UV-induced premutagenic lesions were randomly distributed between the transcribed and nontranscribed strand of the p53 gene. The increase in UV-specific p53 mutation frequency in BCC as a function of age at onset might be due to the persistence in DNA of UV-induced lesions due to age-related decrease of DNA repair efficiency (12).

All p53 mutated tumors apparently retained the wild-type p53 allele since both wild-type and mutant bands were observed in the sequencing gels. Although we cannot completely exclude that this result is affected by the contamination of the tumor samples with normal DNA, two studies (5, 17) have shown that LOH of 17p is a rare event in BCC tumors. The mutated p53 codons of this study coincide with the known cancer-related p53 mutation hot spots (18). The majority lie within exons 7 and 8. As inferred from the crystal structure of the p53 core domain-DNA complex (19), these mutated sites have a crucial role in either contacting DNA (e.g., codons 248 and 278) or stabilizing the protein-DNA interaction (e.g., codons 249 and 282). Moreover, some of them suffer from slow repair (20) of UV-induced damage (e.g., codons 248 and 286). Two tumors presented a known p53 sequence polymorphism at codon 213 (21) which changed an A into a G, leaving unmodified the coding sequence for arginine.

Chromosome Instability. Several studies have shown that BCC occurrence is associated with a typical pattern of chromosome loss. In particular, a high frequency of chromosome 9q loss has been reported (9, 10). The hypothesis has been made that the loss of 9q may be an early genetic event that is followed by inactivation of a single p53 allele (17). We decided to screen the same tumors analyzed for p53 mutations for chromosome 9 instability.

We obtained matched lymphocytes and tumor DNA from 42 of the 45 BCC patients analyzed for p53 mutations. Paired samples of normal and tumor DNA were amplified by PCR for analysis of polymorphic dinucleotide repeats. We used four microsatellite markers to test allelic loss on chromosome 9: D9S157, D9S171, and D9S169 which map at 9p 22–21 and D9S180 which is a marker for 9q22.2. As shown in Fig. 2, both LOH and amplification or deletion of DNA with microsatellite elements were observed independently of the age of BCC onset. LOH was almost exclusively detected on chromosome 9q (25% of the informative cases). We cannot exclude that this phenomenon has been underestimated in our study due to the presence of normal tissue in the tumor biopsy. MI was prevalently observed at 9p loci. When LOH or MI was detected on chromosome 9, the analysis was extended to three other loci on different chromosomes (chromosomes 2 and 10) to ascertain whether the tumor had a RER+ phenotype (22). Extensive MI was detected in 6 of 16 tumor samples analyzed. One example of a tumor with extensive alterations at dinucleotide repeats is shown in Fig. 3. The observed band shifts produced new fragments larger and smaller than the normal alleles. BCC with MI at multiple loci were neither clinically nor pathologically distinguishable from other BCC. LOH was never associated with MI. Although LOH was usually confined to chromosome 9q, patient 12566 showed a wider pattern of chromosomal losses at 9p as well as 2p and 10q markers. This allelotype is very uncommon for BCC (10). Fig. 4 shows LOH for D2S123 and 9p markers detected in this tumor. Interestingly, this nodular BCC belongs to a patient with relatives (both parents and their respective sisters) affected by different types of cancer. Among the tumors characterized by p53 mutation, three of seven informative cases presented LOH of 9q (tumors 6299, 5996, and 4102). In addition, tumor 5996 had a RER+ phenotype.

DISCUSSION

In human BCC we have found chromosome instability as well as mutational changes in the tumor suppressor gene p53. Similarly, in the well-defined multistage mouse skin carcinogenesis model, chromosomal aberrations and mutations in oncogenes and tumor suppressor genes are the hallmarks of tumor progression (reviewed in Ref. 23). UV-specific mutations were identified within the conserved region of the p53 gene. The spectrum of mutations found in this study and in previously reported investigations is in agreement with UVC- and UVB-induced mutational spectrum in both cell culture and mouse systems (reviewed in Ref. 24). The targeting of mutations at pyrimidine runs and the occurrence of tandem mutations at adjacent pyrimidines is the unmistakable “signature” of UV mutagenesis. Another peculiar feature of BCC p53 mutations is that only one allele seems to be inactivated. The maintenance of one Yip allele has been clearly suggested by the hypothesis, a decline of the DNA repair capacity of...
human lymphocytes with increasing age at an estimated rate of 0.61%/year has been reported (12) as well as an age-related increase of mutation rate (1.3%/year) in the housekeeping hprt gene of human lymphocytes (26).

Several studies suggest that p53 mutation occurs early in skin cancer progression (27), even before the appearance of macroscopically visible skin lesions (28, 29). However, BCC developed at a young age rarely (6%) present p53 mutations. The studies conducted in mice (29) indicate that the timing of p53 alterations is dependent on the carcinogenic regimen (carcinogen and/or its application). For example, p53 mutation is a late event in chemically induced skin carcinogenesis (single application of the initiator 7,12-dimethylbenz(a)anthracene followed by chronic application of a tumor promoter; reviewed in Ref. 23), whereas it is an early event following chronic UV exposure (29). This raises the interesting possibility that the sun exposure behavior of young versus older BCC patients is responsible for the differences observed in p53 mutation frequency. A relatively high proportion of young BCC patients reported acute recreational sun exposure during childhood and adolescence, whereas most older BCC patients had a history of occupational exposure. It is possible that chronic UV exposure (as that recorded in older subjects) is necessary not only to initiate the target cells by p53 mutation but, even more importantly, to exert selective pressure on those cells with dysfunctional p53 [and then partially impaired in the production of apoptotic sunburn cells (27)] that will form the tumor. p53 mutations are likely to be induced also in skin cells of young BCC patients, but the pattern of acute UV exposure might be insufficient for the promotion/progression step. In this scenario, factors other than p53 inactivation, e.g., genetic susceptibility, might be responsible for skin cancer formation at an early age.

Chromosome alterations were detected in our BCC samples independently of the age of tumor onset, indicating that this event is a general hallmark of skin cancer progression. The pattern of genomic instability in BCC development is characterized by LOH of 9q (25% of the informative cases) and in some tumors by extensive instability at dinucleotide repeats. LOH on several chromosome arms has been described in atypical keratinocytes from AK (30) which is an early stage in squamous cell carcinoma development, thus suggesting that this phenomenon is an early event in skin cancer progression. AK cells are also characterized by a high frequency of p53 mutations and
the base changes found implicated sunlight as the mutagen (27). The 9q marker that we have used maps to chromosome 9q22–31, which is deleted in a high percentage of familial and sporadic BCC (9–11, 31), further supporting the involvement of tumor suppressor genes localized in this region in BCC development. The gene for NBCCS that maps to the same region has been identified recently (32, 33). This gene is a human homologue of the Drosophila segment polarity gene, patched (ptc). Hereditary mutations in NBCCS patients and somatic mutations in sporadic BCC were identified in the human ptc gene (32–34). These findings suggest that this gene is a strong candidate as a tumor suppressor gene in skin tumorigenesis. LOH at 9p was observed only in one tumor which presented extensive genome instability. A putative tumor suppressor gene, p16/MTS1/CDKN2, which encodes a cell cycle regulator protein, maps to the region of 9p21 (35, 36). Deletions and mutations of this gene have been described at high frequency in several human cancer cell lines, including melanoma, and more rarely in primary tumors (35–38). Our data confirm that LOH of 9p is very uncommon in BCC (31).

MI is associated with several types of hereditary and sporadic tumors. The origin of this genetic event is the functional damage to genes involved in mismatch DNA repair (reviewed in Ref. 39). In our study, 6 of 16 tumors with chromosome 9 instability had a RER phenotype, indicating that malfunctioning of mismatch repair might play a role in skin cancer development. Quinn et al. (40), in their study on the involvement of RER in skin carcinogenesis, reported only 1 case of 47 BCCs analyzed with instability at two chromosome 9 microsatellite markers. However, in the same study, MI was detected in AK from a patient with the Muir-Torre syndrome, indicating that MI is associated with several types of hereditary and sporadic tumors. Further support for the role of RER in skin cancer development comes from studies in melanoma, and more recently in nonmelanocytic skin cancer. Anderson et al. (41, 42) reported that MI is rare in BCC (31).

It was thus suggested that BCC progresses by LOH of 9q and inactivation of a single p53 allele, although one of these two genetic events seems to be sufficient per se to lead to BCC development. BCC is a multifactorial disease in which environmental and host genetic factors conspire to create the tumor pathology. Sunlight exposure is the most important environmental factor in BCC development as shown by the accumulation of typical UV-induced mutations in the p53 gene of late onset tumors. Genetic factors in association with sun exposure behavior in adolescence may play a major role in early BCC onset.

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REFERENCES


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