Low Incidence of p53 Mutations in UVA (365-nm)-induced Skin Tumors in Hairless Mice

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Abstract

Mutations with clear “UVB fingerprints” have been observed in the p53 gene of human nonmelanoma skin tumors and of experimentally UVB-induced murine skin tumors. Although UVA (315–400 nm) radiation is also a complete carcinogen, its contribution to sunlight-induced mutagenesis remains poorly characterized. There is experimental evidence that the production of reactive oxygen species plays a more dominant role with long-wave UVA than with UVB radiation. We have induced skin tumors in hairless mice (n = 42) by daily exposure to long-wave UVA (365-nm) radiation. The incidence of p53 alterations in these tumors is low compared to UVB-induced tumors; positive staining for the p53 protein was observed in only 50% of the tumors, and less than 15% of the tumors showed a mutation in one of the exons 5, 7, or 8 of the p53 gene. The pattern of p53 staining was more irregular and less dense compared to UVB, and the mutations (all C—T) were mainly (six of seven) located at codon 267. Besides a general p53 hotspot, this codon is also the main hotspot for UVB-induced skin tumors in mice. No mutations specific for UVA, i.e., mutations specific for reactive oxygen species, could be detected.

Introduction

The causal relationship between solar UV radiation and skin cancer is substantiated by epidemiological as well as experimental data. Experiments with hairless mice have proven that both UVB (280–315 nm) and UVA (315–400 nm) radiation (also UVA sources that emit only wavelengths >340 nm) can induce skin carcinomas. UVA, comprising in general ~95% of the energy of solar UV radiation, is therefore likely to contribute to sunlight-induced carcinogenicity.

Inactivation of the p53 tumor suppressor gene is the most common genetic alteration observed in human cancers at present (5). Its inactivation by mutation or chromosomal deletion may initiate or propagate tumor formation, depending on both the nature of the DNA damage and the tissue involved. The high incidence of p53 alterations found in human and murine SCCs of the skin indicates that the loss of p53 tumor suppressor function might be a key event in solar UV-induced carcinogenesis (2). Mutation spectra in the p53 gene of skin tumors from mouse and man may help to understand underlying differences between UVA and UVB carcinogenesis. UVB-induced p53 mutations in murine (hairless SKH) skin cancers showed great similarity in frequency, type, and location with p53 mutations in human skin carcinomas, indicating the relevance of the hairless mice model to human skin carcinogenesis (6). The mutation spectra are not entirely identical but share common hotspots in the highly conserved domains of the p53 gene. As is typical for UVB radiation, most mutations occurred at dipyrimidine sites and were C—T and tandem CC—TT transitions. A remarkable difference between sun-induced human skin tumors and UVB-induced murine skin tumors is the lower percentage of C—T transitions and the higher percentage of G—T transversions in human tumors (6, 7). This difference, possibly attributable to the formation of 8-hydroxyguanine by UVA-generated ROS, could reflect the contribution of UVA to human skin carcinogenesis. The data available on UVA-induced mutations are scarce, but differences with UVB have been reported, for example, on the aprt locus of UVA-treated Chinese hamster ovary cells and on the episomal lacZ gene in human cells. In the case of the aprt locus, the percentage of T—G transversions was dramatically higher with UVA compared to UVB treatment (8), whereas UVA irradiation induced many more mutations at A:T base pairs than UVB irradiation in the lacZ gene (9). To investigate whether differences in mutations between UVA and UVB occur in vivo in an endogenous gene that is considered important for skin carcinogenesis, we have investigated changes in p53 at the protein and gene level in UVA-induced murine skin tumors and subsequently compared these results to earlier results obtained with UVB radiation. In this study, we present the first direct evidence of a clear difference in p53 alterations in murine skin tumors that were induced with either UVB or UVA radiation. Surprisingly, no indication of an involvement of ROS could be inferred from the mutations detected in the p53 gene from UVA-induced skin tumors.

Materials and Methods

Tumor Induction. The detailed experimental procedure used for tumor induction by chronic UVA irradiation in albino hairless mice will be published elsewhere (10). In brief, male and female SKH:HR1 mice were irradiated daily for 2 h with a custom-made Philips 365-nm source (mercury arc, rigorously low- and high-cut filtered at 350 and 400 nm, respectively, isolating the 365-nm line). The daily exposure on the dorsal surface of the animals in the different dose groups was 75, 140, or 240 kJ/m2 (340–400 nm). Both papilomas (low prevalence) and SCCs (or precursor lesions) were induced. Median tumor induction times for nonpapillomas in these dose groups were 82, 62, and 54 weeks, respectively. In the 240-kJ/m2 dose group, a lot of mice (14 of 24) were removed from the experiment because scratching marks preceded tumor appearance, and in the 75-kJ/m2 dose group, a lot of mice (17 of 24) died before tumor appearance.

Tumor Preparation and DNA Isolation. After euthanasia, tumors were carefully removed from the mice. Most (65%) of the tumors collected were derived from the 140-kJ/m2 dose group. To prepare tumor tissue for histology (standard H&E staining) and immunohistochemical analysis of p53, one portion of the tumors was fixed in 4% buffered formaldehyde for 18 h at 4°C and stored in 70% ethanol until paraffin embedding, according to routine procedures. The other portion was snap frozen in liquid N2 and stored subsequently at −70°C for molecular analysis. Large tumors were divided in two and processed both ways. Isolation of DNA for mutation analysis was performed by standard methods (11).

p53 Analysis. Immunohistochemical staining with the CM-5 polyclonal antibody (kindly provided by D. Lane) was carried out essentially as described...
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Results

Histopathology and p53 Expression. As described in more detail elsewhere (12), but here we used 1:2500 dilutions of CM-5 for overnight incubations. Also, the genomic DNA amplification by PCR and subsequent mutation analysis by sequencing the amplified p53 gene fragments was carried out as described earlier (12).

Mutations Analysis. As shown in Table 1, sequencing the p53 exons 5, 7, and 8 revealed point mutations in 14% (6 of 42) of the tumors analyzed, which is statistically significantly lower ($\chi^2 = 43.01$, $P < 0.0005$) than the high percentages (53–73%) observed in UVB-induced skin tumors (6). In all six tumors, the same C>G→T:A transition in codon 267 of exon 8 was detected, whereas one tumor showed an additional mutation in codon 173 of exon 5. Around 30% of the mutations found in the p53 gene of UVB-induced tumors are C→T transitions at codon 267; therefore, this codon is considered a hotspot (6). All mutations in UVA-induced tumors were missense mutations resulting in a single amino acid change. The number of UVA-induced tumors that has been analyzed both histopathologically and for p53 mutations is still limited, but from macroscopic appearance and experience it is most likely that the great majority of the p53-mutated tumors are SCCs.

Discussion

In this study, we demonstrate a low incidence of alterations in the p53 gene in UVA-induced murine skin tumors compared to similar UVB-induced tumors. The great majority of the latter (75–90%) have been demonstrated to show immunohistochemically detectable amounts of p53 protein in a large part of the tumor tissue. However, in sections of the UVA tumors, only 50% showed a staining comparable to UVB-induced tumors; even in these tumors, often only a small part of the tumor mass showed this pattern (Fig. 1A). One may speculate that these small regions of p53-expressing cells in these UVB-induced tumors could hardly have contributed to the progression, i.e., clonal outgrowth of the tumors, in contrast to the increased number of p53-stained cells in the UVB-induced tumors. A role for the clonal outgrowth of p53-mutated cells has also been suggested recently for human SCC (13).

The incidence as well as the mutation spectrum of the p53 gene in the UVA-induced skin tumors in the present study is clearly different from data accumulated for UVB-induced nonmelanoma skin tumors of hairless mice (6). In about 60% of the UVB-induced tumors, a point mutation in one of the four conserved domains of the p53 gene is mutation, i.e., clonal outgrowth of the tumors, in contrast to the increased number of p53-stained cells in the UVB-induced tumors. A role for the clonal outgrowth of p53-mutated cells has also been suggested recently for human SCC (13).

Even more striking is the absence of any of the previously described “UVA fingerprints” in the few mutated p53 alleles in the UVA-induced tumors (8, 9). Except for one mutation at codon 173, all mutations are located at codon 267, the major UVB hotspot (6). In about 60% of the UVB-induced tumors, a point mutation in one of the four conserved domains of the p53 gene is found, whereas in only 14% of the UVA-induced tumors described in the present study was a point mutation detectable. These results indicate that point mutations in the p53 gene are by no means a mandatory step in UV(A)-induced skin carcinogenesis.

Even more striking is the absence of any of the previously described “UVA fingerprints” in the few mutated p53 alleles in the UVA-induced tumors (8, 9). Except for one mutation at codon 173, all mutations are located at codon 267, the major UVB hotspot (6). In internal cancers, codon 267 is also a hotspot for mutations, partly due to the spontaneous deamination rate of the methylated cytosine at the CpG site (5). It has been hypothesized that the UVB hotspot originates from strongly enhanced deamination of this cytosine because of its previous (12), but here we used 1:2500 dilutions of CM-5 for overnight incubations. Also, the genomic DNA amplification by PCR and subsequent mutation analysis by sequencing the amplified p53 gene fragments was carried out as described earlier (12).

Histopathology and p53 Expression. As described in more detail elsewhere (10), most (65%) analyzed tumors were collected from the previously (12), but here we used 1:2500 dilutions of CM-5 for overnight incubations. Also, the genomic DNA amplification by PCR and subsequent mutation analysis by sequencing the amplified p53 gene fragments was carried out as described earlier (12).

Results

Histopathology and p53 Expression. As described in more detail elsewhere (10), most (65%) analyzed tumors were collected from the 140-kJ/m2 dose group. On macroscopic appearance, 3 papillomas and 22 nonpapillomas were collected. After histological examination, all nonpapillomas were classified as SCC, or its precursor lesion (actinic keratosis), and only one of the three macroscopically identified papillomas showed some features of malignant growth. Extensive nuclear p53 staining, as detected by immunohistochemistry in the majority of UVB-induced tumors (12), is observed in only one-half of the UVA-induced tumors (13 of 25; Fig. 1). This collection of 13 p53-staining tumors consisted of 11 SCCs and 2 actinic keratoses. When the p53-positive percentage of UVB-induced tumors was compared to that of UVB-induced tumors as presented by Dumaz et al. (6), the difference was statistically significant ($\chi^2 = 6.61$, $P < 0.025$). In the remaining tumors, staining was frequently localized only in small regions of the tumor, confined to basal cells or undifferentiated basal-like cells in proliferative compartments. In general, the accumulation of p53 protein showed a different pattern and appeared less frequently than observed previously in UVB-induced tumors, where we found strongly enhanced levels of p53 protein, visible as extensive homogeneous nuclear staining, in 75–90% of the tumors.

Discussion

In this study, we demonstrate a low incidence of alterations in the p53 gene in UVA-induced murine skin tumors compared to similar UVB-induced tumors. The great majority of the latter (75–90%) have been demonstrated to show immunohistochemically detectable amounts of p53 protein in a large part of the tumor tissue. However, in sections of the UVA tumors, only 50% showed a staining comparable to UVB-induced tumors; even in these tumors, often only a small part of the tumor mass showed this pattern (Fig. 1). One may speculate that these small regions of p53-expressing cells in these UVB-induced tumors could hardly have contributed to the progression, i.e., clonal outgrowth of the tumors, in contrast to the increased number of p53-stained cells in the UVB-induced tumors. A role for the clonal outgrowth of p53-mutated cells has also been suggested recently for human SCC (13).

Table 1 p53 mutations in UVA (365-nm)-induced murine skin tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Exon</th>
<th>Codon</th>
<th>Base substitution</th>
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<tbody>
<tr>
<td>13.1-T1</td>
<td>8</td>
<td>267</td>
<td>Cgt → Tgt</td>
</tr>
<tr>
<td>13.7-T3</td>
<td>5</td>
<td>173</td>
<td>Ctt → Ttt</td>
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<td>13.7-T3</td>
<td>8</td>
<td>267</td>
<td>Cgt → Tgt</td>
</tr>
<tr>
<td>14.3-T2</td>
<td>8</td>
<td>267</td>
<td>Cgt → Tgt</td>
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<td>14.6-T6</td>
<td>8</td>
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<tr>
<td>15.3-T6</td>
<td>8</td>
<td>267</td>
<td>Cgt → Tgt</td>
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</table>

Forty-two tumors were analyzed.

Sequence of the nontranscribed strand (5' → 3') is shown.
presence in a cyclobutane pyrimidine dimer formed on a dipyrimidine sequence, i.e., the CpG site at codon 267, which is preceded by a thymine (14–16). In contrast to the direct formation of cyclobutane of the process of UVA carcinogenesis. Other, yet to be identified, genes and or cellular constituents could be the complete absence of any base transversion in the p53 gene of the p53 gene highly unlikely. However, mutation analysis of larger numbers of in vivo UVA-induced tumors (preferable in more frequently mutated genes) is needed to get further insight into UVA carcinogenesis. 

In conclusion, the p53 gene does not appear to play an equally important role in UVA-driven as in UVB-driven skin carcinogenesis. Other, yet to be identified, genes and or cellular constituents could be more important targets for UVA and, therefore, are more at the core of the process of UVA carcinogenesis.

Acknowledgments

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References

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