Skin tumours induced by narrowband UVB have higher frequency of p53 mutations than tumours induced by broadband UVB independent of Ogg1 genotype

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Different wavelengths of ultraviolet (UV) light have different promoting effects on skin carcinogenesis. Narrowband UVB (NB-UVB) has a single-peak wavelength of 311 nm and is widely used for treating skin diseases. Our previous work showed that, in comparison with conventional broadband UVB (BB-UVB), long-term exposure to NB-UVB induces higher frequency of skin cancer in mice, and it suggested that this is mediated through the formation of cyclobutane pyrimidine dimers (CPDs). To explore whether the frequency of p53 mutations in skin tumours correlates with CPD-induced mutations, we compared the frequency and types of p53 mutations between NB-UVB–induced and BB-UVB–induced malignant skin tumours produced in wild-type and Ogg1 knockout mice, which are deficient in repair of oxidative 8-oxoG (8-oxoG), a DNA damage mediated by reactive oxygen species (ROS). The frequency of p53 mutation was significantly higher in NB-UVB–induced than in BB-UVB–induced tumours in both wild-type and Ogg1 knockout mice. Most of the p53 mutations found were G:C → A:T transitions at dipyrimidine sites in both the NB-UVB– and BB-UVB–exposed groups. However, G:C → T:A mutations caused by 8-oxoG did not increase in Ogg1 knockout mice exposed to either NB-UVB or BB-UVB. Our results strongly suggest that NB-UVB induces highly malignant tumours caused by p53 dipyrimidine mutations through the formation of CPDs.

Introduction

Ultraviolet (UV) light has both harmful and beneficial effects on human skin. It may cause skin cancer, but it is also useful for the treatment of various skin diseases and vitamin D3 photosomeration. Narrowband UVB (NB-UVB), which emits narrow wavelength with a peak at 311 nm, has been widely used to treat various skin diseases, such as psoriasis, atopic dermatitis, vitiligo, and mycosis fungoides; in this respect, it is more effective than conventional broadband UVB (BB-UVB) (1–4) (supplementary Figure S1, available at Mutagenesis Online). Previously, we studied the effects of long-term exposure to NB-UVB or BB-UVB on mouse skin carcinogenesis and showed that continuous NB-UVB exposure induced higher rates of malignant tumours and faster induction of tumours than did BB-UVB. Furthermore, mouse skin exposed to 1 minimal erythema dose (MED) of NB-UVB exhibited significantly more cyclobutane pyrimidine dimers (CPDs) than did the mice skin exposed to 1 MED of BB-UVB–exposed, suggesting that NB-UVB-induced highly malignant tumours may be attributed to the formation of CPDs (5).

The role of the tumour suppressor gene p53 in human and mouse UV-induced skin carcinogenesis has been well studied (6,7). In BB-UVB–induced murine squamous cell carcinoma (SCC) and solar UV-induced human skin cancers, most p53 gene mutations are C → T transition at dipyrimidine sites, a UV-signature mutation (8,9). To determine if the more highly malignant phenotype of NB-UVB–induced skin tumours is associated with higher frequency of p53 mutations, we compared the p53 mutation frequency between malignant skin tumours induced by long-term exposure to NB-UVB and BB-UVB. The Ogg1 gene encodes a repair enzyme that removes the oxidised base 8-oxoG-DNA (8-oxoG) from DNA (10). Among the many reactive oxygen species (ROS)-induced DNA base modifications, 8-oxoG can pair with adenine as well as cytosine during DNA replication (11,12). UV light induces 8-oxoG formation in murine skin, likely through UV-induced ROS (13,14). Previously, we showed that BB-UVB induced more 8-oxoG in the skin of Ogg1 knockout mice than did NB-UVB (5). These data suggest that the more malignant phenotype of NB-UVB–induced skin tumours may be attributed to the formation of CPDs, rather than 8-oxoG. We therefore investigated whether the Ogg1 genotype affects the frequency of p53 mutations in NB-UVB– and BB-UVB–induced skin tumours. If ROS-induced DNA damage is involved in the initiation of UVB-associated skin carcinogenesis, then more 8-oxoG signature mutations of p53 (C → A:T transversions) would be expected in BB-UVB–induced skin tumours of Ogg1 knockout mice (15,16).

We showed in this study that the frequency of p53 mutations was significantly higher in NB-UVB–induced than in BB-UVB–induced tumours in both wild-type and Ogg1 knockout mice. Nearly 70% of all p53 mutations, most of which were G:C → A:T transitions at dipyrimidine sites, were found in the NB-UVB–exposed group. However, the 8-oxoG signature mutations did not increase in Ogg1 knockout mice exposed to either NB-UVB or BB-UVB, indicating little involvement, if any, of 8-oxoG in the induction of p53 mutations during UV-induced skin carcinogenesis. This study has allowed us, for the first time, to compare the spectrum of p53 mutations between NB-UVB–induced and BB-UVB–induced malignant skin tumours, as well as the mutation patterns between the wild-type and Ogg1 knockout phenotypes.
Table I. p53 expression of malignant skin tumours for wild-type and Ogg1 knockout mice

<table>
<thead>
<tr>
<th>Histology</th>
<th>NB-UVBa</th>
<th>BB-UVBb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WTb</td>
<td>KOb</td>
</tr>
<tr>
<td></td>
<td>SCC</td>
<td>Sarcoma</td>
</tr>
<tr>
<td>p53 positivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive, &gt;60%</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Partially positive, 5–60%</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Negative, &lt;5%</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total number</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

aUV source. bOgg1 genotype. cTumours were obtained by chronic exposure of both wavelength UVB and diagnosed histologically as described previously (5, 18). SCC, squamous cell carcinoma.

**Fig. 1.** Expression of p53 in mice skin tumours induced by NB-UVB and BB-UVB irradiation. Various staining patterns of p53 in skin tumours are shown. The positivity of monoclonal antibody PAb240 was described as positive (>60% of cells positive; top panel), partially positive (5–60% positive; middle panel) or negative (<5% positive; bottom panel). Partially positive cells usually show a clonal patch pattern of proliferation of p53-positive cells as indicated by the arrowheads. Subsequent sequence analysis was performed on areas of these clonal patch clusters. Scale bar, 100 µm.

**Materials and methods**

**UV sources**

Banks of six TL 20W/01RS (NB-UVB) and TL 20W/12RS (BB-UVB) fluorescent lamps (Philips, Eindhoven, the Netherlands) were used to irradiate the mice. TL 20W/01RS lamps emit a narrow single peak around 311 nm. TL 20W/12RS lamps emit a continuous spectrum from 275 to 390 nm, with a peak emission at 313 nm; approximately 65% of that radiation is within the UVB wavelength range (supplementary Figure S1, available at Mutagenesis Online). The irradiance was 7.6 J/m²/s for TL 20W/01RS lamps and 3.8 J/m²/s for TL 20W/12RS lamps at a distance of 40 cm, as measured by an IL1400A radiometer/photometer (International Light, Inc., Peabody, MA, USA) and a UVR-305/365D digital radiometer (Tokyo Kogaku Kikai KK, Tokyo, Japan), respectively. MED was determined with the TL 20W/01RS and TL 20W/12RS lamps as 850 mJ/cm² and 250 mJ/cm², respectively, in C5BL/6J mice.

**Animals**

C57BL/6J wild-type mice and Ogg1 knockout mice with the same background were used. Ogg1 heterozygous mice (C5BL/6J, n = 12) were inbred, and their genotypes were determined as described previously (17). The mice were housed under specific pathogen-free conditions, and all animal experiments were conducted according to the Guidelines for Animal Experimentation of Kobe University School of Medicine.

**Detection of p53 gene mutations**

DNA extraction. DNA was extracted from formalin-fixed paraffin-embedded specimens of skin tumours with mutated p53-positive lesions. After identification of p53-positive lesions by immunohistochemical analysis, 8-µm sections of the same but unstained sections were carefully scratched with a clean needle under a light microscope. DNA was extracted with QIAamp® DNA Micro Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s instructions.

DNA amplification. Polymerase chain reaction (PCR) was performed with a PC-816 thermal cycler (ASTEC, Fukuoka, Japan) with a 25-µl total volume of reaction mixture containing 2 µl of genomic DNA sample, 5 µM of each primer (supplementary Table 1, available at Mutagenesis Online), 2.5 mM dNTP Mixture (Takara Bio, Inc., Shiga, Japan), GeneAmp® 10 × PCR buffer and 0.15 µl of 5U/µl AmpliTag® DNA polymerase (Applied Biosystems, New Jersey, NJ, USA). After an initial denaturation at 94°C for 5 min, amplification was followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 45 s, extension at 72°C for 1 min and then final extension at 72°C for 10 min. The PCR products were purified using QIAquick® DNA Purification Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s protocol, and then reamplified using a second pair of nested primers as shown in supplementary Table 1, available at Mutagenesis Online. The amplified products were loaded on 3% LE agarose gels (Nacalai Tesque, Inc., Kyoto, Japan) and were stained with ethidium bromide.

DNA sequencing. Polymerase chain reaction (PCR) fragments were purified using QIAquick® Gel Extraction Kit (QIAGEN GmbH, Hilden, Germany), and 5 µl was added as template to a sequence reaction volume of 15 µl containing 2 µl of each nested primer (supplementary Table 1, available at Mutagenesis Online), 8 µl of BigDye® Terminator V3.1 (Applied Biosystems, California, USA) and were sequenced on an ABI 3730 DNA Sequencer (Applied Biosystems, Uniondale, NY, USA). The sequence data were compared with the human Ogg1 database using the Web database BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) as described previously (20).
Table II. Mutations of p53 gene in the murine skin tumours with NB-UVB and BB-UVB along with Ogg1 genotype

<table>
<thead>
<tr>
<th>UV source</th>
<th>Ogg1 genotype</th>
<th>Mouse no.</th>
<th>Tumour no.</th>
<th>Histology</th>
<th>p53 positivity</th>
<th>Sample*</th>
<th>Sequence†</th>
<th>Base change</th>
<th>Amino acid</th>
<th>Exon</th>
<th>Codon‡</th>
<th>Strand‡</th>
</tr>
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<tbody>
<tr>
<td>NB</td>
<td>WT</td>
<td>290f</td>
<td>1</td>
<td>SCC</td>
<td>PP</td>
<td>1</td>
<td>gtt Cgt → gtt Tgt</td>
<td>TC → TT</td>
<td>Arg → Cys</td>
<td>8</td>
<td>267</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>291f</td>
<td>1</td>
<td>Sarcoma</td>
<td>P</td>
<td>1</td>
<td>aac Cgc → aac Tgc</td>
<td>CC → CT</td>
<td>Arg → Cys</td>
<td>7</td>
<td>242</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>295m</td>
<td>2</td>
<td>Sarcoma</td>
<td>P</td>
<td>1</td>
<td>gtt Cgt → gtt Tgt</td>
<td>TC → TT</td>
<td>Arg → Cys</td>
<td>8</td>
<td>267</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>297f</td>
<td>1</td>
<td>SCC</td>
<td>PP</td>
<td>1</td>
<td>aac Cgc → aac Tgc</td>
<td>CC → CT</td>
<td>Arg → Cys</td>
<td>7</td>
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<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>299f</td>
<td>2</td>
<td>SCC</td>
<td>P</td>
<td>1</td>
<td>gtt Cgt → gtt Tgt</td>
<td>TC → TT</td>
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<td>8</td>
<td>267</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>310f</td>
<td>1</td>
<td>SCC</td>
<td>P</td>
<td>2</td>
<td>CcC → ctc</td>
<td>CC → TC</td>
<td>Pro → Leu</td>
<td>8</td>
<td>294</td>
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<tr>
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<td>Sarcoma</td>
<td>P</td>
<td></td>
<td></td>
<td>tcc gGg → gcc G</td>
<td>G → A</td>
<td>Gly → Glu</td>
<td>8</td>
<td>273</td>
<td>T</td>
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<tr>
<td></td>
<td></td>
<td>302m</td>
<td>1</td>
<td>Sarcoma</td>
<td>PP</td>
<td>2</td>
<td>gtt Cgt → gtt Tgt</td>
<td>TC → TT</td>
<td>Arg → Cys</td>
<td>6</td>
<td>207</td>
<td>NT</td>
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<tr>
<td></td>
<td></td>
<td>309f</td>
<td>1</td>
<td>SCC</td>
<td>PP</td>
<td>2</td>
<td>cTt → aTt</td>
<td>G → A</td>
<td>Splice acceptor Intron 5-6</td>
<td>T</td>
<td></td>
<td></td>
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<tr>
<td>BB</td>
<td>WT</td>
<td>166f</td>
<td>1</td>
<td>SCC</td>
<td>P</td>
<td></td>
<td>gtt Cgt → gtt Tgt</td>
<td>TC → TT</td>
<td>Arg → Cys</td>
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<tr>
<td></td>
<td></td>
<td>169m</td>
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<td>SCC</td>
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<td>2</td>
<td>gtt Cgt → gtt Tgt</td>
<td>TC → TT</td>
<td>Arg → Cys</td>
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<tr>
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<td>154f</td>
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<td>SCC</td>
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<td>1</td>
<td>gtt Cgt → gtt Tgt</td>
<td>TC → TT</td>
<td>Arg → Cys</td>
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<td>207</td>
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<td></td>
<td>gtt Cgt → gtt Tgt</td>
<td>TC → TT</td>
<td>Arg → Cys</td>
<td>8</td>
<td>267</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>155f</td>
<td>1</td>
<td>SCC</td>
<td>PP</td>
<td>1</td>
<td>gtt Cgt → gtt Tgt</td>
<td>TC → TT</td>
<td>Arg → Cys</td>
<td>8</td>
<td>267</td>
<td>NT</td>
</tr>
</tbody>
</table>

*The positivity of p53 expression was defined as shown in Table I. P, positive; PP, partially positive.
†Some tumours had several mutations in same section.
‡Sequence of nontranscribed strand (5′→3′) is shown. Mutated bases are indicated with capital letters.
§Codon numbers are according to Soussi et al. (40).
¶The location of dipyrimidine sequence where the mutation takes place on the transcribed (T) and the nontranscribed (NT) is indicated. (-), not at dipyrimidine site.

USA) and 5 µl of purified distilled water. Both forward and reverse strands were sequenced separately for 25 cycles consisting of denaturation at 96°C for 10 s, annealing at 50°C for 5 s and extension at 60°C for 4 min. Sequence reaction products were finally purified using a DyeEx® 2.0 Spin Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s instructions.

Sequence analysis. Sequence analysis was performed on an ABI PRISM® 310 genetic analyser (Applied BioSystems, Inc., California, CA, USA). Mutations were only accepted when both the forward and reverse sequences demonstrated the characteristic single-peak heterozygous mutant pattern, corresponding to the NCBI Reference Sequence NM_011640.3 (supplementary Figure S2, available at Mutagenesis Online). Control DNA was extracted from non-irradiated tail tissue.

Statistical analysis
Statistical differences were determined using a unpaired t test for the mean number of mutations/number of tumours analysed and a χ² test for the positivity of p53 in skin tumours. P < 0.05 was considered statistically significant.

Results
No difference in p53 expression between NB-UVB–induced and BB-UVB–induced malignant skin tumours
We previously showed that compared with BB-UVB treatment, long-term exposure to 1 MED of NB-UVB induced higher
rates of malignant skin tumours in mice (5). We first examined the expression of p53 by immunohistochemical analysis in previously obtained malignant skin tumours induced by long-term irradiation with NB-UVB or BB-UVB (Figure 1). The expression of mutated p53 was clearly detectable in 36 of 45 malignant tumours examined by using the monoclonal antibody PAb240, which detects cells containing p53 mutations (19) (Table 1). p53 expression was not observed in unexposed normal skin epidermis (data not shown). In the NB-UVB–induced tumours, 94.4% (17 of 18) showed positive expression of mutated p53 protein, whereas 70.4% (19 of 27) of the BB-UVB–induced skin tumours were positive (Table 1). However, the frequencies of positive mutant p53 cells were not statistically different between NB-UVB–induced and BB-UVB–induced tumours, even with the inclusion of Ogg1 genotype (x² test). Because the detection of p53 mutations was unlikely in negative tumours (<5%), which contained only a few positive cells without the clonal patched pattern, further genetic studies were performed only on the 36 tumours that stained positive or partially positive for p53.

Characterization of p53 mutations in tumours from NB-UVB– and BB-UVB–irradiated mice

We directly sequenced p53 PCR products to examine mutations in the 36 positively stained tumours (Table 1) and collected multiple sections from individual p53–positive tissues to look for hetero-mutagenesis. We focussed on exons 5 through 9 of p53 because they contain highly conserved domains and over 90% of p53 mutations in human non-melanoma skin cancers occur in these exons (20, 21). Before analysing the mutations, we confirmed that there were no single nucleotide polymorphisms in the C57BL/6J wild-type background in the analysed areas. In analysing the tumours, we found that 26 of the 36 tumours had ≥1 detectable p53 mutations. There were 38 p53 mutations, all of which were point mutations. The detected mutations resulted in amino acid substitutions, non-sense mutations in exons or mutations in intron sites. Nine of the 16 NB-UVB–induced tumours had double or triple mutations; 7 of these had CGT → TGT mutation at a dipyrimidine site in codon 267. Two of the BB-UVB–induced tumours also had a double mutation, one of which was of the same type as the mutation at codon 267.

Higher p53 mutation frequency in NB-UVB–induced tumours

In wild-type mice, there were 1.78 p53 mutations per tumour in the NB-UVB–exposed group, which was significantly higher than the p53 mutation frequency observed in the BB-UVB–induced tumours (0.80; P < 0.02) (Table 3). This difference remained significant after the numbers of mutations per tumour in wild-type mice and Ogg1 knockout mice were combined (NB-UVB, 1.53; BB-UVB, 0.63; P < 0.001). The most frequent base substitutions in both NB-UVB–induced and BB-UVB–induced tumours were G:C → A:T transitions at dipyrimidine sites, which are a hallmark of UV-induced mutations (22). Ninety-two percent (24 of 26) of mutations in the NB-UVB group and 100% (12 of 12) of mutations in the BB-UVB group were transitions at dipyrimidine sites. Two G:C → T:A and G:C → C:G transversions were found in the NB-UVB group, whereas no transversion was detected in BB-UVB group. These results were surprising because we expected BB-UVB to induce higher rates of G:C → T:A mutations and this tendency to be greater in Ogg1 knockout mice. However, our results revealed that the mutation rates induced by BB-UVB and NB-UVB were similar. Our results also demonstrated that the highly malignant phenotype of NB-UVB–induced skin tumours was closely associated with higher frequency of transition-type mutations at p53 dipyrimidine sites, possibly resulting from the formation of CPDs rather than 8-oxoG. Furthermore, no transversions were found in either NB-UVB– or BB-UVB–induced tumours in Ogg1 knockout mice, although G:C → T:A transversions were expected, similar to the 8-oxoG-induced G:C → T:A substitutions observed in Escherichia coli (23) (Tables 2 and 3). All base changes at dipyrimidine sites were single-base substitutions, and no tandem-base changes such as CC → TT or TT → CC were detected. All but three mutations occurred in non-transcribed strands: 92.3% (24 of 26) in the NB-UVB tumour group and 91.7% (11 of 12) in the BB-UVB group.

Next, we analysed if there were mutation hot spots in p53 specific to NB-UVB–induced tumours in comparison with BB-UVB–induced tumours. Our results revealed that codon 267 was the most frequent p53 mutation in both the NB-UVB and BB-UVB tumour groups (Figure 2), in accord with the results of previous studies (8, 24–26). Mouse codon 267 corresponds to codon 273 of human p53, which is in a conserved domain and has DNA-binding activity, like codon 248, one of the mutation hot spots in human skin cancers (8). So far, the codon 267 mutation has been reported only in BB-UVB–induced murine skin cancers, but not in other types of mouse cancers, confirming the role of UV-induced photolesions at this codon (8, 25–29). Codon 272, which was also mutated in the NB-UVB–induced tumour group, is another mutational hot spot in BB-UVB–induced tumours (8). Mutations in codon 267 occur at the CpG-associated dipyrimidine sites. Many p53 mutation hot spots are reported to be located at CpG sites in mammalian skin cancers (30); this might be because solar UVB induces CPD formation at methylated cytosines at a higher frequency owing to the UV absorption shift to the higher wavelength region at methylated cytosines (31). Our data show that...
57.7% (15 of 26) of all mutations in the NB-UVB tumour group were C → T transitions at CpG sites, whereas in the BB-UVB group, 91.7% (11 of 12) of the mutations were located at dipyrimidine sites associated with CpG. In this study, we also found that 10 mutations in the BB-UVB tumour group were similarly distributed to the p53 hot spots in the NB-UVB group (Figure 2). Collectively, our results provide strong supporting evidence for the hypothesis that highly malignant skin tumours induced by NB-UVB are closely associated with higher frequencies of p53 mutations at dipyrimidine hot spots resulting from CPD formation.

Discussion

We have previously shown that the clinical use of NB-UVB phototherapy for various skin diseases might be more associated with a greater risk of developing skin cancer than conventional BB-UVB therapy (5). In the same report, we suggested that the higher carcinogenic activity of NB-UVB could be attributed to the greater formation of CPDs. The main goal of the present study was to find the underlying genetic cause of the association between NB-UVB and skin carcinogenesis. We found that 1 MED of NB-UVB produced more UV-signature mutations than did BB-UVB at dipyrimidine sites of p53, which was strongly suggestive of higher amount of CPD or 64PP formation. Given the evidence from our previous immunofluorescence study that the amounts of 64PP produced were not significantly different between 1 MED NB-UVB and BB-UVB (5), we conclude that NB-UVB–induced mutations at dipyrimidine sites are attributable to the formation of CPDs rather than 64PP. Mouret et al. (32) also examined the distribution of dipyrimidine CPD and 64PP in BB-UVB–irradiated human skin, where most of the photoproduct was TT-CPD, followed by TC-CPD and TC-64PP which were next in abundance, whereas lower amount of CT-CPD, CC-CPD and TT-64PP were present. CT-64PP and CC-64PP were below the limit of detection. CPD lesions were also predominantly induced in whole human skin exposed to UVA radiation. More than 70% of CPDs produced were TT-CPD. In addition, neither 64PP nor Dewar valence isomers were able to be detected. On the other hand, in BB-UVB–irradiated human skin, TT-CPD was produced in a 10-fold higher yield than the TT-64PP. TC-CPD was also generated in a 2-fold higher yield than TC-64PP (32). These data also support the contribution of CPD formation, rather than 64PP formation, to carcinogenesis.

Another significant feature of the p53 mutations detected in this study was that their pattern of base change mutations was not affected by the Ogg1 genotype with either UVB source. The NB-UVB results are concordant with our previous results in which the accumulation of 8-oxoG in the skin was not different between wild-type and Ogg1 knockout mice after a single exposure to NB-UVB. Our previous studies showed that BB-UVB induced a higher frequency of skin tumours in Ogg1 knockout mice (18) and higher levels of 8-oxoG than did NB-UVB (5). From these findings, we speculated that the higher skin tumorigenesis in Ogg1 knockout mice might be due to mutations in other genes.

Table III. Characterization of all p53 gene mutations detected in the murine skin tumours with NB-UVB and BB-UVB of Ogg1 genotype

<table>
<thead>
<tr>
<th></th>
<th>NB-UVB⁺&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th>BB-UVB⁺&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>WT (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>KO (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Total (%)</td>
<td>WT (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>KO (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Total (%)</td>
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<td>Total number of mutations</td>
<td>16</td>
<td>10</td>
<td>26</td>
<td>4</td>
<td>8</td>
<td>12</td>
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<td>Total tumours analysed</td>
<td>9</td>
<td>8</td>
<td>17</td>
<td>5</td>
<td>14</td>
<td>19</td>
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<tr>
<td>Average number of mutations/tumour</td>
<td>1.78*</td>
<td>1.25</td>
<td>1.53**</td>
<td>0.80*</td>
<td>0.57</td>
<td>0.63**</td>
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<tr>
<td>Nontranscribed</td>
<td>15 (94)</td>
<td>9 (90)</td>
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<td>3 (75)</td>
<td>8 (100)</td>
<td>11 (92)</td>
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<tr>
<td>Transcribed</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>4 (100)</td>
<td>8 (100)</td>
<td>12 (100)</td>
</tr>
<tr>
<td>Others&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<sup>a</sup>UV source.
<sup>b</sup>Ogg1 genotype.
<sup>c</sup>Transitions and transversions refer to the types of point mutations at dipyrimidine sites.
<sup>d</sup>Others include mutations occurring at absence of dipyrimidine sites.

*P < 0.02, **P < 0.001.
to skin tumours induced with MED of BB-UVB. Third, the more tumorigenic higher dose of BB-UVB might cause more mutations of \( p53 \), with the result that the ROS-induced mutation patterns could be seen for both genotypes of mice. However, the MED is the standard measurement in clinical phototherapy and the only option for a comparative study between NB-UVB and BB-UVB, which limits the dose for induction of\( p53 \) mutations with the C57BL/6J mouse strain. Fourth, there is the possibility that 8-oxoG could be repaired by nucleotide excision repair (NER). Several previous reports showed that oxidative DNA damages were repaired by NER in \( E. \) coli and Saccharomyces cerevisiae (35, 36), suggesting that oxidative DNA damage might be a substrate for NER. The fifth and the most plausible theory for not detecting the ROS-induced mutation patterns with BB-UVB in \( Ogg1 \) knockout mice is that the 8-oxoG accumulation itself alters the cell microenvironment to favour the formation of skin tumours (37). This might contribute more to the development of BB-UVB-induced skin tumours in \( Ogg1 \) knockout mice than do the \( p53 \) mutations.

Another possible explanation for the higher rate of skin cancer in the NB-UVB–irradiated group is that CPD could induce immunosuppression. UV-induced immune suppression has been shown to play an important role in the development of skin cancer, and CPD formation mediates UV-induced immune suppression (38). On the other hand, we have to consider the difference of repairability for CPDs following UVB between humans and mice. CPDs in human keratinocytes were well removed compared with mice keratinocytes (39). Therefore, given that higher NB-UVB energy level induces a high frequency of \( p53 \) mutations, the beneficial effects of NB-UVB for treating disease must be balanced with its carcinogenic side effects.

In conclusion, our genetic study clearly suggests that the higher incidence rate of NB-UVB–induced malignant tumours than BB-UVB–induced tumours is associated with higher mutations at dipyrimidine sites in \( p53 \), mediated by the formation of CPDs. The \( Ogg1 \) genotype did not affect the patterns of \( p53 \) mutation in skin tumours induced by either BB-UVB or NB-UVB. Our study provides further information on the genetic effects of different UV wavelengths on skin tumorigenesis.

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Conflict of interest: The authors declare no conflicts of interest.

**Supplementary data**

Supplementary Table 1 is available at Mutagenesis Online. Supplementary Figures S1 and S2 are available at Mutagenesis Online.

**References**

Higher frequency of \( p53 \) mutation by narrowband UVB


