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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学位論文の内容要旨

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

ナローバンドUVB照射誘導による皮膚発癌はブロードバンドUVB照射によるものよりも高率に*p53*遺伝子の変異を起こしそれらは*Ogg1*遺伝型とは関係なく起こる

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

INTRODUCTION

Different wavelengths of ultraviolet (UV) light have different promoting effects on skin carcinogenesis. Narrowband UVB (NB-UVB) has a narrow band emission peaking at 311 nm and is widely used for treating skin diseases. Our previous work showed that long-term exposure to NB-UVB induces higher frequency of skin various cancer in mice than conventional broadband UVB (BB-UVB), and suggested that this is mediated through the formation of cyclobutane pyrimidine dimers (CPDs). The role of the tumour suppressor gene *p53* in human and mouse UV-induced skin tumours has been well studied. 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. 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 oxidized base 8-oxoG-DNA (8-oxoG) from DNA. UV light induces 8-oxoG formation in murine skin, likely through UV-induced ROS. Previously, we showed that BB-UVB induced more 8-oxoG in the skin of *Ogg1* knockout mice than did NB-UVB. 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 tumours.

MATERIAL AND METHODS

Tumour induction by NB-UVB and BB-UVB irradiation : For induction of skin tumourigenesis, C57BL/6J wild-type (WT) mice and *Ogg1* knockout (KO) mice with the same background were placed 40 cm below the bank of six TL 20W/01RS (NB-UVB) and TL 20W/12RS (BB-UVB) fluorescent lamps and their shaved backs were irradiated 3 times per week for 40 weeks.

Immunohistochemistry : Forty-five malignant skin tumour specimens were included for the immunohistochemical detection of p53 expression. The sections were incubated with monoclonal antibody

against p53, PAb240. p53 expression was described as positive (>60%), partially positive (5%–60%), or negative (<5%) as the average % of positive cells for ten 100-μm^2 fields in one slide. Monoclonal antibody PAb240 detects only mutated p53 protein in murine skin tissues. Therefore, only the positive and partially positive malignant skin tumours were subjected to *p53* mutation analysis.

Detection of p53 gene mutations : DNA was extracted from formalin-fixed paraffin-embedded specimens of skin tumours with mutated p53-positive lesions using QIAamp® DNA Micro Kit. DNA was amplified and PCR products were purified then re-amplified once again. PCR fragments were purified and was added as template to a sequence reaction volume with BigDye® Terminator V3.1. Sequence reaction products were purified and sequence analysis was performed on an ABI PRISM® 310 genetic analyser. Mutations were only accepted when both the forward and reverse sequences demonstrated the characteristic mutant pattern, corresponding to the NCBI Reference Sequence NM_011640.3.

Statistical analysis : Statistical differences were determined using an un-paired *t* test for the mean number of mutations/number of tumours analysed, and χ^2 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: 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. 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 (χ^2 test).

Characterization of *p53* mutations in tumours from NB-UVB- and BB-UVB-irradiated mice : We found that 26 of the 36 tumours had ≥1 detectable *p53* mutations. There were 38 *p53* mutations, all of which were point mutations. 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 WT mice, there were 1.78 *p53* mutations per tumour in the NB-UVB-exposed group, which was significantly higher than those observed in the BB-UVB-induced tumours (0.80; $P < 0.02$). This difference remained significant after the numbers of mutations per tumour in WT and *Ogg1* KO mice were combined (NB-UVB 1.53; BB-UVB 0.63; $P < 0.001$). The most frequent base substitutions in both groups were G:C→A:T transitions at dipyrimidine sites, which are a hallmark of UV-induced mutations. Ninety-two per cent (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. Furthermore, no transversions were found in either NB-UVB- or BB-UVB-induced tumours in *Ogg1* KO mice. All base changes at dipyrimidine sites were single-base substitutions. All but 3 mutations occurred in nontranscribed strands: 92.3% (24 of 26) in the NB-UVB group and 91.7% (11 of 12) in the BB-UVB group.

DISCUSSION

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 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, we conclude that NB-UVB-induced mutations at dipyrimidine sites are attributable to the formation of CPDs rather than 64PP.

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. Our previous studies showed that BB-UVB induced a higher frequency of skin tumours in *Ogg1* knockout mice and higher levels of 8-oxoG than did NB-UVB. However, we found that all the BB-UVB-induced mutations of *Ogg1* KO mice were at dipyrimidine sites, indicating that ROS-mediated mutations in the *p53* gene do not contribute to BB-UVB-induced skin tumours in these mice.

In conclusion, our genetic study clearly suggests that 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 tumourigenesis. 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.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第2360号	氏　名	Flandiana Yogianti
論文題目 Title of Dissertation	Skin tumours induced by narrowband UVB have higher frequency of *p53* mutations than tumours induced by broadband UVB independent of *Ogg1* genotype ナローバンドUVB 照射誘導による皮膚発癌はブロードバンドUVB照射によるものよりも高率に*p53* 遺伝子の変異を起こしそれらは *Ogg1* 遺伝型とは関係なく起こる		
審査委員 Examiner	主　査 Chief Examiner　堀田 博 副　査 Vice-examiner　林 祥剛 副　査 Vice-examiner　栗 健		

（要旨は１，０００字～２，０００字程度）

Different wavelengths of ultraviolet (UV) light have different promoting effects on skin carcinogenesis. Narrowband UVB (NB-UVB) has an emission peak at 311 nm and is widely used for treating skin diseases. Long-term exposure to NB-UVB was shown to induce higher frequency of skin cancer in mice than conventional broadband UVB (BB-UVB), which is thought to be mediated through the formation of cyclobutane pyrimidine dimers (CPDs). The role of the tumor suppressor gene *p53* in UV-induced skin tumors in human and mice has been well studied. In BB-UVB-induced murine squamous cell carcinoma and solar UV-induced human skin cancers, most *p53* gene mutations are C→T transition at dipyrimidine sites, a UV-signature mutation.

In order to determine whether the more highly malignant phenotype of NB-UVB-induced skin tumors is associated with higher frequency of *p53* mutations, this candidate compared the *p53* mutation frequency between malignant skin tumors induced by long-term exposure to NB-UVB and BB-UVB. The *Ogg1* gene encodes a repair enzyme that removes the oxidized base 8-oxoG-DNA (8-oxoG). UV light induces 8-oxoG formation in murine skin, likely through UV-induced reactive oxygen species. It was previously shown that BB-UVB induced more 8-oxoG in the skin of *Ogg1* knockout mice than did NB-UVB. The data suggest that the more malignant phenotype of NB-UVB-induced skin tumors may be attributed to the formation of CPDs, rather than 8-oxoG. Therefore, this candidate investigated whether the *Ogg1* genotype affects the frequency of *p53* mutations in NB-UVB- and BB-UVB-induced tumors. The result obtained showed that 94.4% (17/18) and 70.4% (19/27) of the skin tumors induced by NB-UVB and BB-UVB, respectively, showed positive staining of mutated p53 protein. By DNA sequencing analysis, this candidate also found that 26 of the 36 tumors had ≥1 detectable *p53* mutations. There were 38 *p53* mutations, all of which were point mutations. The most prominent hotspot was found on codon 267. In the wild-type mice, there were significantly more mutations in the *p53* gene per tumor in the NB-UVB group than in the BB-UVB (1.78 vs. 0.80). This difference remained significant when the numbers of mutations per tumor in the wild-type and *Ogg1* knockout mice were combined (1.53 vs. 0.63). The most frequent base substitutions in both groups were G:C→A:T transitions at dipyrimidine sites, which are a hallmark of UV-induced mutations, with 92% (24/26) and 100% (12/12) of mutations in the NB-UVB and BB-UVB groups, respectively, being transitions at dipyrimidine sites. Thus, the results obtained in this study collectively suggest that higher incidence rate of skin tumors induced by NB-UVB compared to BB-UVB is associated with higher mutations at dipyrimidine sites in the *p53* gene, which was likely mediated by the formation of CPDs.

This candidate, having completed her studies on UVB-induced skin tumors, with a specialty in UVB-induced genetic mutations, and having advanced the field of knowledge in the area of *p53* gene mutations induced by NB-UVB and BB-UVB in the wild-type and the *Ogg1* knockout mice, is hereby recognized as having qualified for the degree of Ph.D. (Medicine).