Mutation Research 791 (2015) 42–52

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Development and validation of a new transgenic hairless albino mouse as a mutational model for potential assessment of photocarcinogenicity

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ARTICLE INFO

Article history:
Received 5 June 2015
Received in revised form 31 July 2015
Accepted 1 August 2015
Available online 7 August 2015

Keywords:
Transgenic Hairless albino mouse
gpt-Delta mouse
Spi⁻ selection assay
Mutant frequency
Mutation frequency

ABSTRACT

Short-term phototoxicity testing is useful in selecting test agents for the longer and more expensive photocarcinogenesis safety tests; however, no validated short-term tests have been proven reliable in predicting the outcome of a photocarcinogenesis safety test. A transgenic, hairless, albino (THA) mouse model was developed that carries the *gpt* and *red/gam* [Spi⁻] genes from the *gpt* delta mouse background and the phenotypes from the SKH-1 mouse background to use as a short-term test in lieu of photocarcinogenesis safety tests. Validation of the THA mouse model was confirmed by exposing groups of male mice to sub-erythemal doses of ultraviolet B (UVB) irradiation for three consecutive days emitted from calibrated overhead, Kodacel-filtered fluorescent lamps and measuring the mutant frequencies (MFs) in the *gpt* and *red/gam* (Spi⁻) genes and types of mutations in the *gpt* gene. The doses or irradiation were monitored with broad-spectrum dosimeters that were calibrated to a NIST-traceable standard and cumulative CIE-weighted doses were 20.55 and 41.0 mJ/cm^2 (effective). Mice were sacrificed 14 days after the final UVB exposure and MFs in both the *gpt* and *red/gam* genes were evaluated in the epidermis. The exposure of mice to UVB induced significant ten- to twelve-fold increases in the *gpt* MF and three- to five-fold increases in the Spi⁻ MF over their respective background MF, $26 \pm 3 \times 10^{-6}$ and $9 \pm 1 \times 10^{-6}$. The *gpt* mutation spectra were significantly different between that of the UVB-irradiated and that of non-irradiated mice although the mutation spectra of both groups were dominated by C → T transitions (84% and 66%). In mice exposed to UVB, the C → T transitions occurred almost exclusively at dipyrimidine sites (92%), whereas in non-irradiated control mice, the C → T transitions occurred at CpG sites (86%). These results suggest that the newly developed THA mice are a useful and reliable model for testing UVB-induced mutagenicity in skin tissue. The application of this model for short-term prediction of solar-induced skin carcinogenicity is presently under investigation.

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1. Introduction

Skin cancers are the most prevalent and rapidly increasing malignancies among Caucasians in the United States and worldwide, with an estimated 900 thousand to 1.2 million new cases diagnosed each year in the United States alone [1,2]. Skin cancers are associated with substantial morbidity and cost, as well as a relatively small but substantial mortality [3]. Treatment costs for skin cancers are estimated at more than $500 million annually in the United States alone [4]. In addition, studies have indicated that skin cancer survivors experience an increased risk for subsequent new malignancies [5,6]. The primary pathogenic factor for the development of skin cancer in the general population is overexposure to the ultraviolet radiation (UVR) in sunlight, and this association is strongly supported by epidemiology studies [7,8].

The sun emits UVR with wavelengths that are shorter than visible light (400–760 nm), but longer than X-rays (≤10 nm). Solar UVR can be divided into three regions: UVC (200–290 nm); UVB

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http://dx.doi.org/10.1016/j.mrgentox.2015.08.001
1383-5718/Published by Elsevier B.V.

(290–315 nm); and UVA (315–400 nm) [9]. UVA, containing the longest wavelengths of the three, is subdivided further into two wavelength regions: UVA1 (340–400 nm); UVA2 (315–340 nm) [10]. With the shortest wavelengths of the three UVR components, UVC is absorbed and greatly attenuated almost entirely by atmospheric gases, namely ozone, and does not reach the Earth. Therefore, the cutaneous effects resulting from solar UVR exposure are attributed to UVB and UVA [10]. Both the U.S. Department of Health and Human Services and the World Health Organization have identified UVR as a proven carcinogen and consider UVR to be the main cause of non-melanoma skin cancers and a primary contributor to melanoma skin cancer [11].

When UVR and visible light reach the skin, one part is reflected and the other part is absorbed into the various skin layers. Wavelengths in the UVB range are absorbed primarily by epidermal cellular components, e.g. DNA and proteins; whereas, the longer wavelengths of UVA penetrate further into the skin and reach the basal cells of the epidermis and even the dermis [12]. The different UVR components have distinct mutagenic properties [13]. The mutagenic effects of solar UVB irradiation are mainly mediated by the direct absorption of photons by DNA, and the *cis-syn* cyclobutane pyrimidine dimers (CPDs) and the pyrimidine-(6, 4)-pyrimidone photoproducts (6-4PPs) are the most representative DNA lesions [14]. These lesions increase the incidence of C → T single and CC → TT double transitions and tandem base substitutions at dipyrimidine sites in the *p53* tumor suppressor gene in human and rodent skin squamous cell and basal cell carcinomas [13,15–17]. UVA, which penetrates the skin more deeply than UVB, generates CPDs in DNA with a large predominance of TT CPDs and can promote the formation of oxidized DNA bases [18–20].

The photocarcinogenesis safety test is a laboratory study that involves repeated intercurrent exposures of simulated solar light and a test article [21]. The traditional test uses the hairless (*hr*/*hr*) mouse model, which is immunocompetent and comparable with other mice in DNA damage repair mechanisms, and specified dose levels of UVR that are designed to provide an adequate response range within a study duration of one year [22]. Short-term phototoxicity testing is useful in selecting test agents for the longer and more expensive photocarcinogenesis safety tests; however, no validated short-term tests have been proven reliable in predicting the outcome of a photocarcinogenesis or photococarcinogenesis safety tests.

The transgenic *in vivo* genotoxicity assays have the unique ability to measure mutations in reporter genes engineered into the DNA of several commonly used animal models and are useful for the risk assessment of genotoxic agents that cause cancer [23]. The *gpt* delta mouse is an established transgenic model [24]. This model carries the *gpt* transgene that detects point mutations, as well as the *red/gam* transgene that detects deletion mutations [25]. In the mutation assay using the transgenic *gpt* delta mouse, 6-thioguanine (6-TG) selection and Spi$^-$ (sensitive to P2 interference) selection are employed for the efficient detection of point mutations and deletions, respectively [26]. The 6-TG selection uses the *gpt* gene of *Escherichia coli* as a reporter gene to detect base substitutions and frameshift mutations. The Spi$^-$ selection takes advantage of the fact that wild-type λphages display the Spi+ phenotype, so only mutant λ Spi$^-$ phages, lacking both *gam* and *redBA* genes, can form plaques that grow in P2 lysogens of *E. coli*. The simultaneous inactivation of both the *gam* and *redBA* genes is induced by deletions in the region [27].

We have developed a transgenic mouse model named THA that carries the *gpt* and *red/gam* [Spi$^-$] from the *gpt* delta mouse background and the hairless, albino phenotypes from the SKH-1 mouse background to use as a short-term test in lieu of photocarcinogenesis tests. In this paper, we report our results on the validation of the THA transgenic mouse model by testing the relative sensitivities and specificities of the two loci (*gpt* and *red/gam* of Spi$^-$) as mutational targets following exposure of the animal model to UVB.

2. Materials and methods

2.1. Animal source, care, environment

Male and female *Gpt* delta mice (C57BL6/J) were provided by Takehiko Nohmi (NIHS, Tokyo, Japan) in order to establish a breeding colony at the National Center for Toxicological Research (NCTR, FDA, Jefferson, AR). Male and female Crl:SKH-1 (*hr*$^-$/*hr*$^-$) BR hairless mice were obtained from Charles River Laboratories (Wilmington, MA). The care of animals and all animal experimental procedures were performed in accordance with a study protocol approved by the Institutional Animal Care and Use Committee (NCTR). Mice, three weeks of age and older, were group-housed (4 same sex mice per cage) in polycarbonate rodent cages with hardwood chip bedding and micro-isolator bonnets. The environment of the animal rooms was set to maintain a 12 h light cycle, temperature of 22 ± 4 °C, relative humidity of 40–70%, and air changes of 10–15/h. The mice were provided NIH-41-irradiated pellets and Millipore-filtered drinking water *ad libitum*.

2.2. Generation of transgenic hairless albino mouse lines

Transgenic hairless albino (THA) mice were developed by crossing the transgenic, homozygous *gpt* delta male mice (C57BL6/J) with SKH-1 hairless female mice. Male F_1 progeny were backcrossed with SKH-1 hairless female mice to generate hairless, albino pups (Fig. 1) and the hairless, albino backcross progeny was screened for the presence of *gpt* using Real-Time-PCR (RT-PCR) as described below.

2.3. Real-time polymerase chain reaction analysis

The genotype of the hairless, albino pups was determined in DNA (1–2 μg) that was extracted from toe clips of 2–4 day old pups using DNeasy tissue kits (Qiagen, Valencia, CA) in accordance with the manufacturer's procedure. Toe clips provide a painless, perma-

Fig. 1. Pups showing hairless and albino (white) and hairless and pigmented (black) phenotypes. Hair loss is progressive and it starts from their face and pups become completely hairless within two weeks of their birth.