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SKHIN/Sprd, a new genetically defined inbred hairless mouse strain for UV-induced skin carcinogenesis studies

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Abstract

Strains of mice vary in their susceptibility to ultra-violet (UV) radiation-induced skin tumors. Some strains of hairless mice (homozygous for the spontaneous Hr^{hr} mutation) are particularly susceptible to these tumors. The skin tumors that develop in hairless mice resemble, both at the morphologic and molecular levels, UV-induced squamous cell carcinomas (SCC) and their precursors in human. The most commonly employed hairless mice belong to the SKH1 stock. However, these mice are outbred and their genetic background is not characterized, which makes them a poor model for genetic studies. We have developed a new inbred strain from outbred SKH1 mice that we named SKHIN/Sprd (now at generation F31). In order to characterize the genetic background of this new strain, we genotyped a cohort of mice at F30 with 92 microsatellites and 140 single nucleotide polymorphisms (SNP) evenly distributed throughout the mouse genome. We also exposed SKHIN/Sprd mice to chronic UV irradiation and showed that they are as susceptible to UV-induced skin carcinogenesis as outbred SKH1 mice. In addition, we proved that, albeit with low efficiency, inbred SKHIN/Sprd mice are suitable for transgenic production by classical pronuclear microinjection. This new inbred strain will be useful for the development of transgenic and congenic strains on a hairless inbred background as well as the establishment of syngeneic tumor cell lines. These new tools can potentially help elucidate a number of features of the cutaneous response to UV irradiation in humans, including the effect of genetic background and modifier genes.

Keywords

ultraviolet radiation; skin carcinogenesis; mouse model; hairless mice

Introduction

Epithelial tumors of the skin can be induced in mice by application of chronic exposure to ultra-violet (UV) radiation. In both haired and hairless mice, skin tumors develop in a reproducible and predictable stepwise fashion, evolving from foci of epithelial hyperplasia into pre-malignant papillomas and ultimately into malignant squamous cell carcinoma

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(SCC) and spindle cell carcinomas (1–3). Different inbred strains and outbred stocks vary in their susceptibility to UV-induced skin tumors. Some strains and stocks of hairless mice (homozygous for the spontaneous Hr^{hr} mutation) are particularly susceptible to these tumors (2). The skin tumors that develop in these hairless mice resemble, at the morphologic and molecular levels, UV-induced papillomas and SCC in humans, including comparable alteration in p53 and cell cycle–associated proteins (2, 4, 5). The most commonly employed hairless mice in UV photobiology belong to the SKH1 albino stock (2, 6, 7). However, these mice are outbred and their genetic background is not characterized, which makes them a poor model for genetic studies. The SKH1 mouse (Crl:SKH1-*hr*) marketed by Charles River Laboratories (Wilmington, MA) was originally obtained from a commercial supplier in New York City via Temple University. This outbred stock carries the hairless allele (*hr*) at the hairless gene (*Hr*). The *hairless* (Hr^{hr}) mutation is autosomal recessive and contains a modified polytropic retrovirus stably integrated into intron 6 of the *Hr* gene, resulting in aberrant splicing of over 95% of the *Hr* transcripts (8, 9). In total, more than 25 *Hr* alleles are currently known in the mouse, including spontaneous, chemically induced, and targeted mutations (10, and Mouse Genome Informatics database resource). Mutations in orthologs of the mouse *Hr* gene have been identified in other species, including humans (11–13). The history and uses of hairless mice in skin research (including available strains) as well as the structure and functions of the *Hr* gene have been reviewed in detail elsewhere (9, 10, 14–18). In this study, we present a new genetically defined inbred strain derived from outbred SKH1 mice. Similar to SKH1 outbred stock, SKHIN/Sprd mice exhibit high susceptibility to UV-induced skin carcinogenesis. This new isogenic strain, homozygous for the Hr^{hr} allele, has been characterized for more than 200 genetic markers (microsatellites and single nucleotide polymorphisms), the *H2* haplotype, and a few gene polymorphisms of interest. This new mouse model will constitute an excellent tool for the identification of genes that modify susceptibility to UV-induced skin carcinogenesis in the mouse and for examining the contribution of the Hr^{hr} mutation to the carcinogenesis process. In addition, to expand the utility of this strain, we analyzed the efficiency of transgenic production and showed that it is possible to develop transgenic animals on this isogenic hairless genetic background.

Materials and methods (Data S1)

Mice and inbreeding process

Outbred SKH1 mice were purchased from Charles River Laboratories (Wilmington, MA) as SKH1-Elite mice (Crl:SKH1-Hr^{hr}, strain code 477). SKH1 and SKHIN/Sprd mice were maintained under specific pathogen free (SPF) conditions in our AAALAC International accredited Animal Facilities at The University of Texas M.D. Anderson Cancer Center, Smithville and Houston, Texas. All procedures were in compliance with the Public Health Service Guide for the Care and Use of Laboratory Animals (National Research Council, 2010). To start the inbreeding process, a single ancestral breeding pair was randomly selected from the purchased SKH1 outbred mice. From the F1 generation, mice have been maintained by brother-to-sister mating. To avoid creating sublines we kept the number of generations tracing back to a common ancestor to five. When mice reached F30 we selected 10 non-littermates to extract genomic DNA from tail biopsies using standard procedures. These DNA samples were used for genotyping.

UV carcinogenesis and tissue collection

The UV apparatus used for the chronic carcinogenesis consisted of eight Westinghouse FS20 sunlamps, an IL-1400 radiometer, and an attached UVB photometer. The spectral irradiance for the UV lamps was 280–400 nm, 80% of which was in the UVB region and 20% in the UVA region. The peak intensity of the radiation source was 297 nm; the fluence at 60 cm from the dorsal surface of the mouse was 0.48–0.50 $mJ/cm^2/s$. The mice were

placed in individual compartments in a plastic cage on a rotating base to abrogate any differences in fluence across the UV radiation bulbs. The cage was covered by a UVB-transparent lid that filtered out the small amount of UVC radiation emitted from these lamps. Mice were exposed to 90 mJ/cm^2 three times per wk; the dose was increased by 10% per wk until the dose reached 175 mJ/cm^2. Outbred SKH1 (n = 12) and inbred SKHIN/Sprd (n = 7, at F27) were chronically treated with UV. In an independent chronic study, mice were exposed initially to 100 mJ/cm2 per day, and the exposures were increased by 20% per wk for the first 4 wks and remained at this level for the duration of the study. Mice were irradiated 5 days per wk. Statistical significance was determined by calculation of p values using Wilcoxon Rank Sum test.

Results and discussion

Development of the new SKHIN/Sprd inbred strain

We started the inbreeding process from newly purchased outbred SKH1 mice in 2001 and attained generation F30 with one line in 2010. Although some hairless females did not nurse their litters, we could complete the inbreeding without the need of foster mothers and using exclusively hairless (homozygous) breeding pairs. We nicknamed this strain as SKHIN/Sprd (SKH1 INbred), a homophone for skin, and registered the strain name “SKHIN” at the Mouse Genome Informatics (accession ID MGI: 5141760). Two lines were lost before F10, most likely due to inbreeding depression. We characterized the breeding performance of 33 females at F30 and found the following average values: (i) number of pups per female/lifetime = 24.9; (ii) litter size = 5.8; (iii) number of litters = 4.3; (iv) age at first productive mating = 10.9 wk; and (v) age at last productive mating = 28.4 wk. Considering the total number of pups produced per female in a lifetime, the overall fecundity of the SKHIN mice is not apparently different from other classical inbred strains like DBA/2J, C3H/HeJ, or 129X1/J (19), other than a later age for the first productive mating.

UV-induced skin carcinogenesis studies

In a comparative study involving inbred SKHIN (at generation F27) versus outbred SKH1, the difference in tumor incidence was not statistically significant between the two groups at the end of the study, reaching a plateau of 85% at 32 wk (Fig. 1a). Skin tumors in both groups did not regress. The tumor multiplicity was also similar between the inbred and outbred groups. The SKHIN/Sprd mice (n = 7) developed tumors earlier (16 wk), with an average of 1.2 tumors per mouse by 20 wk of UV irradiation and 2.6 ± 1.9 tumors per mouse at the end of the study at 34 wk (Fig. 1b). In SKH1 outbred (n = 12), the first tumors arose at 20 wk, and tumor multiplicity was 3.7 ± 2.7 tumors per mouse at 34 wk (p=0.38, not statistically significant). We measured the diameters of the skin tumors at the termination of the experiment and assigned them to size categories. The category 1–4 mm represented 89% and 83% of the tumors for the outbred and inbred mice, respectively. A pathological characterization of the tumors was conducted at the termination of the experiment on representative samples. Table 1 shows a comparison of the tumors induced by UV radiation in each group of mice.

In an independent chronic UV carcinogenesis study we challenged inbred SKHIN/Sprd (F29) and outbred SKH1 with an alternative protocol (see Materials and Methods). The tumor multiplicity was higher with this protocol, but not statistically significant between the two groups. At 34 wk, inbred SKHIN/Sprd (n = 8) developed 8.25 ± 2.6 tumors per mouse and outbred SKH1 (n = 9) 7.8 ± 4.1 tumors per mouse (data not shown and Fig 1c). A typical SCC obtained after chronic exposure to UV in SKHIN/Sprd inbred mice is shown in Fig. 1d.

Genetic background characterization by microsatellite and SNP markers

In order to perform a genetic characterization of the new inbred strain, we selected 92 (multi-allelic) microsatellites (also known as simple sequence length polymorphisms, SSLPs) and 140 (bi-allelic) SNPs known to be polymorphic between FVB/NJ and C57BL/6J. We chose these markers because many targeted mutations and transgenes are carried on these two inbred backgrounds, therefore polymorphic markers between SKHIN/Sprd and these strains will be essential for the development of congenic strains on a hairless SKHIN/Sprd background. Out of the 92 SSLP loci analyzed, 68 (74%) were polymorphic (more than 2 bp difference) between SKHIN/Sprd and C57BL/6J and 50 (54%) with FVB/NJ. On the other hand, the SNP allelotyping showed that out of the 140 SNP loci analyzed, 2 (1.4%) were still heterozygous (residual heterozygosity) and 138 (98.6%) homozygous among the SKHIN/Sprd samples. From these, 96 (69.6%) were polymorphic with C57BL/6J and 42 (30.4%) with FVB/NJ. The SSLP and SNP allele distribution is given in Supporting Information Data S2.

H2 haplotype and other gene-specific genotypes

We genotyped 10 randomly selected SKHIN/Sprd mice (F30) to determine the *H2* haplotype as well as a few polymorphisms and mutations found in albino strains that we considered relevant to the genetic characterization of the strain. Using a PCR-RFLP technique we could determine that SKHIN/Sprd mice carry the $H2^{b}$ haplotype, the same haplotype described for C57BL/6 mice. With regard to the gene-specific genotypes, we first confirmed by PCR and direct sequencing that both SKH1 and SKHIN/Sprd mice carry the classical Hr^{hr} allele with the retroviral insertion (10). Second, we analyzed the *Ptch1* gene polymorphism described by Wakabayashi and colleagues for FVB/N mice (20). Interestingly, SKHIN/Sprd mice carried the SCC susceptible allele ($Ptch^{FVB}$/asparagine) of this carboxy-terminal polymorphism in the *Ptch1* gene. Third, we sequenced a region of the *Skint1* gene previously described to harbor a G-T transversion that leads to a premature stop codon in the FVB/NTac substrain. This mutation can affect the regulation of cutaneous inflammation (21). We confirmed that the SKHIN/Sprd strain does not carry the mutant allele. Finally, we genotyped the SKHIN/Sprd mice for the presence of the spontaneous retinal degeneration 1 mutation ($Pde6b^{rd1}$) found in FVB/N, C3H/He, CBA/J and other inbred strains, causing severe retinal degeneration and vision loss. We confirmed that SKHIN/Sprd mice carry the wild-type allele of the *Pde6b* gene.

Efficiency of transgenic production

In an effort to determine the best age for superovulation for the SKHIN/Sprd mice, we examined oocyte production in response to PMSG/HCG treatment in females at 3.5, 5, 7, 9, 9.5, 11 and 11.5 wk of age. We counted the numbers of total and injectable embryos at day 0.5 after fertilization. To avoid under-counting fertilized eggs due to small, or underdeveloped/late developing pronuclei, we incubated the eggs overnight to confirm fertilization. All oocytes progressing to the 2-cell stage after overnight incubation were considered fertilized. All surviving oocytes remaining at the 1-cell stage were considered unfertilized. As shown in Table 2, females at 3.5 wk of age had the greatest number of immediately injectable embryos per female, and thus, would be the optimal age for superovulation for pronuclear injection. However, the average number of fertilized embryos per female is not significantly different among the various ages, suggesting that, at 3.5 wks, the fertilized embryos mature more quickly than at later ages. Thus, we found that SKHIN/Sprd females produce “slow-maturing” eggs after normal fertilization, with a high percentage of embryos showing small or poorly defined pronuclei until late in the day 1. An inbred strain containing the hairless mutation should be a valuable resource for generating transgenic models. Thus, we examined whether we could produce transgenic founders using

SKHIN/Sprd mice. Injections were initially performed using a DNA construct that contained an easily assayable GFP marker. During the first injection attempt, 286 embryos were collected, 51 were chosen for injection, 21 were transferred, and only 3 pups were born, none of which were transgenic either by GFP illumination or by DNA analysis. In a second injection attempt, 45 embryos were transferred, and 4 pups were born, again, none of which were transgenic. An additional injection was performed using a proven construct with 45% founder rate. For this injection, 15 embryos were transferred and 3 pups were born, of which 1 was transgenic (6.6% efficiency). This transgenic founder proceeded to pass the transgene on to 50% of the offspring. Thus, SKHIN/Sprd mice can be used to produce transgenic animals, although the success rate appears to be highly dependent upon the DNA construct used.

UV-induced skin carcinogenesis in the mouse (hairless and hairy strains) is a relevant experimental model for human skin carcinogenesis (22–24). Although a few hairless inbred strains are available (e.g., HRS/J and SKH2/J), outbred SKH1 mice are the most widely used in dermatologic research, including acute photobiologic responses and skin carcinogenesis (2, 5, 7). These albino and immunocompetent mice allow for ready manipulation of the skin, application of topical agents, and UV irradiation, as well as easy visualization of the cutaneous response. Two limitations of the SKH1 mice are the relatively uncharacterized genetic background and its outbred status, which precludes syngeneic grafts. To address this shortcoming, we developed a new inbred strain from outbred SKH1 mice. The new inbred hairless strain that we characterized in this study will potentially constitute a valuable mouse model for acute photodamage and photocarcinogenesis studies. Moreover, using SKHIN/Sprd mice to test chemopreventive agents in UV-induced carcinogenesis should help reduce the number of mice needed, since inbred strains offer more uniform and repeatable results than outbred stocks (25). Based solely on the different standard deviations in tumor multiplicity in our experiments with the outbred SKH1 and inbred SKHIN/Sprd, a power analysis indicates that a hypothetical experiment designed to detect significant differences between control and treatment groups would require two times more outbred than inbred mice, with a power of 80%. Congenic strains carrying targeted alleles (knockouts and knockins) as well as transgenes (including *cre* constructs) on this new hairless inbred background can now be developed through classical or marker-assisted (26) methods. This is important because the high susceptibility of the SKH1 and SKHIN/Sprd mice to UV carcinogenesis is probably due to a combination of the genetic background (modifier genes) and the presence of the Hr^{hr} mutant allele. For example, moving the Hr^{hr} allele onto a classical inbred strain like C57BL/6 (selected for the International Knockout Mouse Consortium to mutate all protein-coding genes in the mouse) will most likely result in a different susceptibility to UV-induced carcinogenesis, compared with SKH1 and SKHIN/Sprd. In addition, microinjecting a transgene construct into the pronuclei of SKHIN/Sprd embryos will be a direct way to generate a transgenic line on a hairless inbred background. This advantage could be eventually extended to the new zinc finger nuclease technology (27) that requires pronuclear microinjection, but not ES cell manipulation. Finally, it is important to point out a potential drawback of using hairless mice in photocarcinogenesis. Both outbred SKH1 and inbred SKHIN/Sprd mice carry the same disruptive mutation on the *Hr* gene (10). It was recently shown that, when homozygous, this mutant allele (Hr^{hr}) confers susceptibility to UV-induced skin tumors in SKH1 mice acting as a repressor of the NFKB1 (NF-kappaB) signaling pathway (28), raising some concerns about the clinical relevance of using hairless mouse for carcinogenesis studies. Although there are some weaknesses in using hairless mice, the new inbred strain described here provides a useful model for the study of photodamage and photocarcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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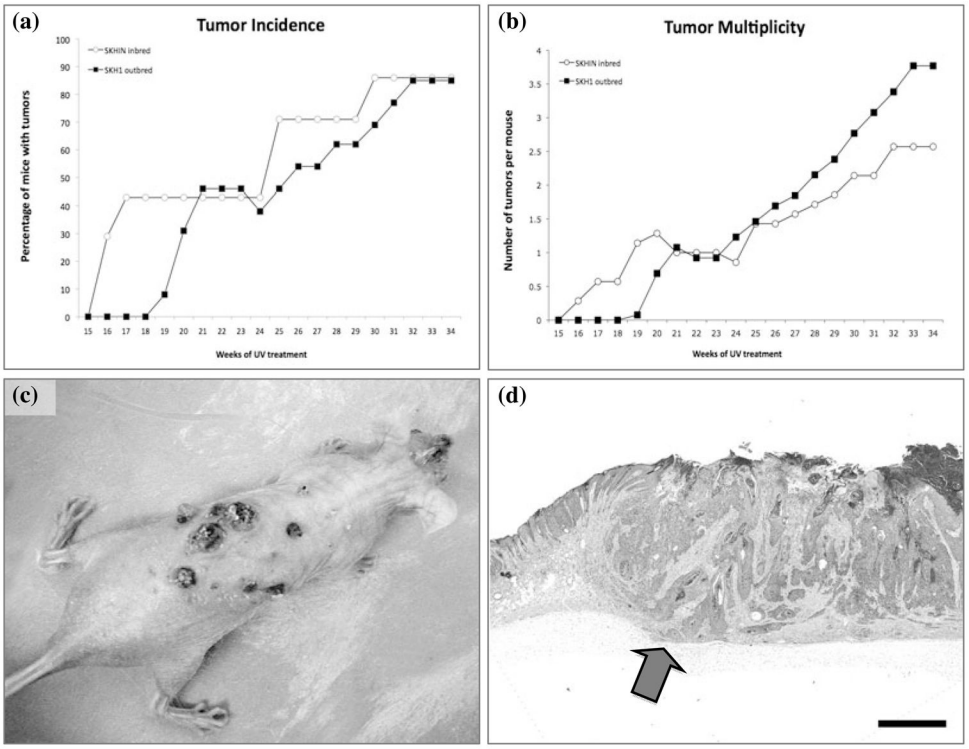

Figure 1. UV-induced carcinogenesis studies in SKHIN/Sprd mice

(**a**) Tumor incidence in SKHIN/Sprd mice reached a plateau of 85% at 30 wk, followed by outbred SKH1 mice at 32 wk (p=1.0, non-parametric Wilcox Rank Sum). (**b**) Tumor multiplicity after 34 wk of UV treatment reached 2.6 ± 1.9 tumors per mouse in SKHIN/Sprd mice (n = 7), compared with 3.7 ± 2.7 tumors per mouse for the outbred SKH1 (n = 12) (p=0.38, Wilcoxon Rank Sum test). Inbred SKHIN/Sprd mice (❍); Outbred SKH1 mice (■). (**c**) Gross image of skin tumors in a SKHIN/Sprd mouse after 34 wk (second experiment). (**d**) Representative hematoxylin and eosin staining of a typical large SCC that developed in SKHIN/Sprd mice. Note the atypical squamous epithelium and keratin pearl formation and how the tumor penetrates the *panniculus carnosus* (arrow). Scale bar, 1 mm. Weekly tumor counts were performed following the appearance of the first tumor and continued until termination of the experiment at 34 wk. The tumor data are expressed both as multiplicity (i.e. mean number of tumors per mouse), and incidence (i.e. percent mice with tumors). Tumors were removed at the end of the experiment, fixed in formalin and processed for histological evaluation of tumor type (papilloma, SCC, or spindle cell carcinoma).

Table 1

Types of tumors induced by UV radiation (representative samples)

	# Mice	# Paps	# miSCC	# SCC	Spindle cell	Total tumors
SKH1 (outbred)	6	1	6	5	1	13
SKHIN (inbred)	7	2	4	8	0	14

Paps, papillomas; miSCC, microinvasive SCC; SCC, squamous cell carcinoma*

* classification of tumors as decribed in reference 29

Table 2

Response to superovulation at various ages.

Age (wks)	# females	Total # embryos	Injectable embryos	Inj. embs./ female	Unfertilized at day 0.5 PC	Abnormal	Dead	Unfertilized at day 1.5PC	Total fert. embs.	Fert. embs./ female
3.5	6	198	98	16.3	93	1	6	57	134	22.3
5	7	204	58	8.3	130	15	1	45	142	20.3
7	7	229	53	7.6	159	14	3	45	167	23.9
9	6	222	66	11.0	145	4	7	44	167	27.8
11	6.5	242	33	5.1	198	7	4	92	139	21.4

SKHIN females were tested at ages 3.5 through 11 weeks to determine their response to superovulation. Parameters were then averaged per female. To examine total fertilized embryos, all oocytes whose fertilization status could not be determined at day 0.5 PC were incubated and then assessed for development to the two-cell stage the following day.

Note: We had the loss of one oviduct of an 11 week female; thus, the number of animals in this age group is 6.5, rather than 7.