Carcinogenesis vol.29 no.10 pp.1950–1954, 2008
doi:10.1093/carcin/bgn160
Advance Access publication July 18, 2008

A role for ultraviolet radiation immunosuppression in non-melanoma skin cancer as evidenced by gene–environment interactions

Marleen M.Welsh[1], Margaret R.Karagas[2], Katie M.Applebaum[1], Steven K.Spencer[3], Ann E.Perry[4] and Heather H.Nelson[5,*]

[1]Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA, [2]Section of Biostatistics and Epidemiology, Department of Community and Family Medicine, Norris Cotton Cancer Center, Dartmouth Medical School, Lebanon, NH 03756, USA, [3]Section of Dermatology, Department of Medicine and [4]Department of Pathology, Dartmouth Hitchcock Medical Center, Lebanon, NH 03756, USA and [5]Division of Epidemiology and Community Health, Masonic Cancer Center, University of Minnesota, Minneapolis, MN 55455, USA

*To whom correspondence should be addressed. Tel: +1 612 626 9887; Fax: +1 612 626 4842;
Email: hhnelson@umn.edu

The genotoxic effects of ultraviolet (UV) radiation are well-known causes of skin cancers; however, UV radiation also suppresses the immune system, decreasing the body's surveillance for tumor cells. In experimental systems, UV radiation immunosuppression is at least partially mediated through urocanic acid (UCA), an UV radiation-absorbing molecule in the stratum corneum. We tested the hypothesis that genetic variation in the histidase gene (*HAL*), which catalyzes the formation of UCA in the skin, modifies risk of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) in a population-based study (914 BCC, 702 SCC and 848 controls). We observed no evidence of a main gene effect for the *HAL* I439V polymorphism (rs7297245) and BCC or SCC. However, we found a *HAL* genotype–sunburn interaction in association with BCC (P for interaction = 0.040) and SCC (P for interaction = 0.018). A *HAL* genotype–SCC association was observed primarily among women (odds ratio = 1.5, 95% confidence interval 1.1–2.2), and among women, we found an interaction between *HAL* genotype and oral contraceptive use on SCC risk (P = 0.040). The variant *HAL* allele likewise appeared to modify the SCC risk associated with glucocorticoid steroid usage (P for interaction = 0.0004). In conclusion, our findings are a first step in determining the genetic underpinnings of UV immune suppression and have identified important new genetic interactions contributing to the etiology of skin cancer.

Introduction

Ultraviolet (UV)-B radiation is known to contribute to skin cancer progression through direct DNA damage, but also has a role in reducing immune responses that are essential to preventing malignancy. As UV-B waves can only penetrate the first few layers of the epidermis, there must exist a photoreceptor in the outermost layers of skin that can be activated by UV and then signal elsewhere in the body to stimulate wider immunosuppression. Experimental data implicate both DNA damage and urocanic acid (UCA) as potential photoreceptors for UV immunosuppression. We chose to focus on the role of UCA as a photoreceptor in this study. Histidine ammonia-lyase (*HAL*, histidase) catabolizes the amino acid L-histidine to *trans*-UCA, which then accumulates in the outermost layers of the skin. Upon exposure of the epidermis to UV light, specifically UV-B, *trans*-UCA can photoisomerize to *cis*-UCA. It is this isomer, *cis*-UCA, that has been shown to mimic the effects of UV-B-mediated immunosuppression *in vitro* and *in vivo*, including alteration in tumor antigen presentation by Langerhans cells and release of neuropeptides, histamine and cytokines that are critical to the immunosuppressive signaling cascade from peripheral nerves and mast cells (1–6). Also, administration of antibodies to *cis*-UCA has been shown to abrogate UV-induced immunosuppresssion of contact hypersensitivity responses (7).

Data from both human and animal studies indicate that distinct 'UV-susceptible' and 'UV-resistant' populations exist and respond differently to equal doses of UV radiation, implicating a genetic component to this phenotype (8). Human studies determined that ~35–40% of normal Caucasians are considered UV-susceptible (n = 34) (9). A similar prevalence was found in darker skinned populations (n = 18), indicating that UV-induced immunosuppression occurs irrespective of pigmentation (10). One study of skin cancer patients classified 92% of its population (n = 12) as UV susceptible (9), suggesting a link between UV-induced immunosuppression and cancer. Experiments using mouse models found that the C57BL/6 strain is highly susceptible to UV-induced immunosuppression (11) and exhibits high liver histidase activity (12). In contrast, the UV-resistant C3H/HeJ strain (13) has low histidase activity, exhibiting 2-fold lower activity than the C57BL/6 mice (12). These limited experimental data indicate a correlation between histidase activity and degree of UV-related immunosuppression in mice.

There is only one known non-synonymous coding polymorphism in the human *HAL* gene, an isoleucine (ATT) to valine (GTT) switch in amino acid 439 (14). The functional relevance of this change is unknown. We hypothesize that variation in this gene that initiates the UV-B-induced immune signaling cascade might increase risk of non-melanoma skin cancers (NMSC). Therefore, we examined the prevalence of the *HAL* I439V single-nucleotide polymorphism (SNP) (rs7297245) in a Caucasian population in New Hampshire, as part of a larger case–control study of NMSCs. Further, as continued messenger RNA synthesis is required for maintenance of histidase levels (15), and transcription is regulated by both glucocorticoids and estrogen (16–18), we also tested for potential interactions with both intense UV exposure as well as these transcriptional activators of *HAL*.

Materials and methods

Study population

Newly diagnosed cases of histologically confirmed basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) in New Hampshire were identified using an incident survey established through the collaboration of dermatologists, dermatopathologists and pathology laboratories throughout the state and bordering regions from 1 July 1993 to 30 June 1995 (series 1) and from 1 July 1997 to 30 March 2000 (series 2) (19). The study design for the New Hampshire Health Study has been described previously by Karagas *et al.* (19). Briefly, eligibility criteria for cases were as follows: (i) between 25 and 74 years of age; (ii) a listed telephone number and (iii) spoke English. All eligible SCC cases and a ratio of approximately two to one BCC cases in series 1 and one to one ratio in series 2 were selected to take part in the study. The BCC cases were randomly sampled in order to ensure representativeness of age, sex and anatomic site for all incident BCCs within New Hampshire. Controls aged 25–64 years were identified from the New Hampshire State Department of Transportation files and those aged 65–74 years were obtained from enrollment lists from the Center for Medicaid and Medicare Services. Potential controls were frequency-matched on age and gender to the combined distribution of case groups. We have found that 98.5% of cases ages 25–64 years had a valid driver's license at the time of interview, and 98% of those ages 65–74 were enrolled in Medicare (20).

A personal interview was conducted with consenting cases and controls, with ~80% of cases and 72% of controls agreeing to participate. The interviews, usually conducted in the participant's home, covered demographic factors, pigmentation characteristics, sun exposure and sensitivity and other factors (19). Blood draws and/or buccal samples were obtained from cases

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; NMSC, non-melanoma skin cancer; OC, oral contraceptive; OR, odds ratio; SCC, squamous cell carcinoma; SNP, single-nucleotide polymorphism; UCA, urocanic acid; UV, ultraviolet.

© The Author 2008. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org

Downloaded from https://academic.oup.com/carcin/article/29/10/1950/2476412 by guest on 21 August 2022

and controls from both phases of the study for DNA analysis. Approximately 85% of subjects consented to providing a DNA sample, 90% of which were blood derived. All study protocol and materials were approved by the Dartmouth College Committee for the Protection of Human Subjects, and all participants provided informed consent.

HAL genotyping

DNA was extracted from peripheral blood lymphocytes of consented cases and controls using Qiagen Genomic DNA extraction kits (Valencia, CA). Genotyping for the histidase I439V SNP (rs7297245) was performed by polymerase chain reaction–restriction fragment length polymorphism analysis. A 333 bp fragment of exon 16 of the *HAL* locus was amplified using primers as described previously (14). Genomic DNA was amplified in a 25 μl reaction with 100 pmol of each primer, 0.2 mM of each deoxyribonucleotide triphosphate, 1X polymerase chain reaction buffer with 15 mM $MgCl_2$ (Applied Biosystems, Foster City, CA) and 1.25U recombinant *Taq*Gold DNA polymerase (Perkins Elmer, Waltham, MA). Polymerase chain reaction conditions were a 5 min incubation at 95°C, followed by 40 cycles of 94°C for 1 min, 54°C for 40 s and 72°C for 40 s; this was followed by a 10 min extension at 72°C. Then 10 μl of amplified products were subject to restriction digest for 3 h at 65°C with 3U *TasI* (Fermentas, Hanover, MD), in a total volume of 50 μl. Digestion products were separated by electrophoresis on 3% agarose gels, at 225V for 40 min. The variant form of the polymorphism (G) interrupts the *TasI* consensus sequence, leaving the full 333 bp product. The wild-type A form results in a single cleavage of the DNA into 161 and 172 bp products that appear as one band upon electrophoresis. Positive and negative controls were included to demonstrate proper amplification and complete digestion. In total, genotyping was attempted on 2523 samples, but only 2464 were successfully genotyped (failure rate = 2.3%).

For quality assurance purposes, 10% of blood and buccal samples were used as integrated duplicates, to which researchers were blinded at the time of genotyping. The genotypes of the embedded duplicates were compared afterward with that of the original samples to calculate the genotyping error rate. Error rate for rs7297245 were <1%.

Statistical analysis

Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for models examining the *HAL* I439V polymorphism as a risk factor for NMSC were obtained using unconditional logistic regression. All models were adjusted for age at diagnosis (continuous), gender, skin pigmentation and lifetime number of severe sunburns. Additionally, models calculating the risk associated with oral contraceptive (OC) use controlled for level of education (three-level variable: less than college, college and longer than college). Self-reported sunburn history was described as the lifetime number of painful sunburns that last 3 or more days. For all analyses, tertile cutoffs were determined based on the distribution within controls, creating groups of no (0), low (one to three) and high (≥4) burns. 'Ever' use of glucocorticoid steroids was defined as having been prescribed any oral form of corticoid steroids for at least 1 month. This variable was self-reported and included betamethasone, dexamethasone, fludrocortisones, hydrocortisone, methylprednisolone, prednisolone, prednisone and triamcinolone. Ever use for OCs was defined as having taken OCs for 3 months or longer. Starting in September of 1995, the questionnaire asked about OC use; from that time on, ~99% of women reported their OC intake.

Pigmentation score was calculated using a multivariate confounder score (21,22), as described earlier (23). Briefly, the pigment score included reaction to first hour of intense sun (burn only, burn then tan and tan only), skin reaction following repeated sun exposure (freckle/no tan, mild tan then peel, moderate tan and deep tan), hair (red, blonde, light brown and black/dark brown), eye (blue/gray, green/hazel and brown/black) and skin color (medium or light). Beta-values for each of these factors were calculated using a single unconditional logistic regression model, with combined risk of BCC and SCC as the outcome. A summation score for each subject was then calculated using these beta-values. A higher pigment score was indicative of a fairer complexion, whereas darker pigmented Caucasians had lower pigment scores. Based on the distribution of pigment score within controls, tertiles were formed and used for all analyses.

In most cases, *HAL* genotype was treated as a binary variable with dominant inheritance, such that the heterozygotes (AG) and homozygous variants (GG) were collapsed into one category. Hardy–Weinberg equilibrium was calculated in controls using a X^2 test. To test for statistical interaction between *HAL* genotype and various exposures, models were generated that included separate main effect terms for each of the variables (i.e. genotype and the exposure of interest) as well as an additional cross product interaction term. The log likelihood was then compared with the log likelihood from a similar model that did not contain the cross product term. If the exposure and the genotype were binary, a one degree of freedom X^2 test was performed; if the genotype was tertiary and the exposure binary, a two degree of freedom test was performed. All tests were two sided, and a *P*-value of ≤0.05 was considered significant.

Genotype data are missing for only 2.3% of the original population tested, and missing genotype data were not associated with case status. Of those 2464 subjects that were successfully genotyped, 115 were missing data on pigmentation or sunburns. This means that <5% of our population was excluded when controlling for these factors using logistic regression. There is a high level of concordance between subjects missing pigment data and those missing sunburn data. Of the 115 subjects missing either pigment or sunburn data, 74 (64.3%) are missing both. Ten subjects (8.7%) are missing pigment data only and 31 subjects (27.0%) are missing sunburn data only. There was no significant difference between the distribution of age, sex or *HAL* genotype between those missing data and those not. Therefore, we believe the missing data would not alter the results observed in this study significantly and were excluded from analysis.

Results

A total of 2464 participants were successfully genotyped for the I439V SNP in histidase (848 controls, 914 BCC cases and 702 SCC cases). The frequency distributions for age, sex and other known risk factors for NMSC, such as pigmentation and lifetime number of sunburns, are shown in Table I. The average age of participants was 61.3 years (SD = 10.6) for controls, 58.7 years (SD = 11.2) for BCC and 64.2 years (SD = 8.7) for SCC. In all groups, there were more men than women. BCC and SCC cases were more likely to have experienced severe sunburns and be more fair skinned than controls.

Within controls, the prevalence of the *HAL* variant allele was 22.1% and met Hardy–Weinberg equilibrium criteria (*P* = 0.90). The variant allele prevalence was slightly higher in case groups (BCC: 23.1% and SCC: 23.6%). After controlling for age, gender, skin pigmentation and lifetime number of severe sunburns, the ORs for both SCC (AG: OR = 1.1, 95% CI 0.9–1.4; GG: OR = 1.2, 95% CI 0.7–1.9) and BCC (AG: OR = 1.0, 95% CI 0.8–1.3; GG: OR = 1.2, 95% CI 0.8–1.9) were minimally elevated (Table II).

Modification by UV-related factors

While there were no overall main gene effects, *HAL* genotype modified the effects of sunburn history on BCC and SCC risk. There was no significant difference in risk among those with no burns or with low burns (one to three lifetime severe sunburns) so these two categories were collapsed into a single referent group (data not shown). For both BCC and SCC, those who reported four or more lifetime severe sunburns had increased odds of disease, and this effect was greater in those of the variant *HAL* genotype. Compared with the wild-type-no/low sunburn group, a 2.7-fold increased odds in BCC (95% CI 1.3–5.8) and a 3.4-fold increase in odds in SCC (95% CI 1.6–7.5) were observed among those who were homozygous variant and reported

Table I. Selected characteristics of BCC and SCC patients and controls collected by the New Hampshire Health Study from 1993 to 2000

	Controls, $n = 848$	BCC, $n = 914$	SCC, $n = 702$
Age (SD)	61.3 (±10.6)	58.7 (±11.2)	64.2 (±8.7)
Sex			
Male	512 (60.4%)	509 (55.7%)	450 (64.1%)
Female	336 (39.6%)	405 (44.3%)	252 (35.9%)
Pigment score[a]			
Dark	266 (33.0%)	153 (17.3%)	114 (16.8%)
Medium	275 (34.1%)	281 (31.7%)	206 (30.3%)
Fair	266 (33.0%)	453 (51.1%)	360 (52.9%)
Lifetime severe sunburns (no.)[b]			
No (0)	261 (32.5%)	219 (24.6%)	170 (25.1%)
Low (1–3)	263 (32.8%)	230 (25.8%)	170 (25.1%)
High (≥4)	278 (34.7%)	441 (49.6%)	338 (49.9%)

[a]Pigment score data missing for 41 controls, 27 BCC cases and 22 SCC cases.
[b]Sunburn data missing for 46 controls, 24 BCC cases and 24 SCC cases.

Downloaded from https://academic.oup.com/carcin/article/29/10/1950/2476412 by guest on 21 August 2022

Table II. Association of *HAL* SNP I439V and its interaction with sunburns in SCC and BCC in New Hampshire Caucasians collected by the New Hampshire Health Study between 1993 and 2000

HAL genotype	BCC			SCC		
	AA	AG	GG	AA	AG	GG
OR (95% CI)[a]	Referent	1.0 (0.8–1.3)	1.2 (0.8–1.9)	Referent	1.1 (0.9–1.4)	1.2 (0.7–1.9)
Cases/controls	526/484	298/269	54/41	395/484	235/269	41/41
History of severe sunburns[b]						
No/low (0–3)						
OR (95% CI)	Referent	0.9 (0.7–1.2)	0.9 (0.5–1.6)	Referent	1.0 (0.7–1.3)	0.8 (0.4–1.4)
Cases/controls	271/304	144/182	25/31	202/304	117/182	17/31
High (≥4)						
OR (95% CI)	1.3 (1.0–1.7)	1.6 (1.2–2.2)	2.7 (1.3–5.8)	1.5 (1.1–1.9)	1.8 (1.3–2.5)	3.4 (1.6–7.5)
Cases/controls	255/180	154/87	29/10	193/180	118/87	24/10
	Interaction P-value = 0.040			Interaction P-value = 0.018		

[a]ORs adjusted for gender, age at diagnosis, pigmentation and lifetime number of severe sunburns.
[b]ORs adjusted for gender, age at diagnosis and pigmentation.

Table III. Association of oral glucocorticoid use and *HAL* SNP I439V with BCC and SCC in New Hampshire Caucasians collected by the New Hampshire Health Study between 1993 and 2000

Oral glucocorticoids[a]	BCC		SCC	
	AA	AG/GG	AA	AG/GG
Never				
OR (95% CI)	Referent	1.0 (0.8–1.3)	Referent	1.0 (0.8–1.3)
Cases/controls	467/437	321/290	339/437	236/290
Ever				
OR (95% CI)	0.8 (0.5–1.4)	2.3 (0.9–5.6)	1.1 (0.7–1.9)	6.6 (2.8–15.8)
Cases/controls	34/36	21/7	31/36	30/7
	Interaction P-value = 0.055		Interaction P-value = 0.0004	

[a]ORs adjusted for gender, age at diagnosis, pigmentation and lifetime number of severe sunburns.

Table IV. Association of estrogen and *HAL* SNP I439V with BCC and SCC in New Hampshire Caucasians collected by the New Hampshire Health Study between 1993 and 2000

	BCC		SCC	
	AA	AG/GG	AA	AG/GG
All women[a]				
OR (95% CI)	Referent	1.1 (0.8–1.5)	Referent	1.5 (1.1–2.2)
Cases/controls	235/198	152/115	135/198	105/115
OCs[b]				
Never				
OR (95% CI)	Referent	0.9 (0.5–1.5)	Referent	1.1 (0.6–1.9)
Cases/controls	67/79	39/53	63/79	42/53
Ever				
OR (95% CI)	1.1 (0.7–1.9)	1.8 (1.0–3.3)	1.5 (0.8–2.7)	3.7 (1.9–7.5)
Cases/controls	92/70	60/28	49/70	47/28
	Interaction P-value = 0.11		Interaction P-value = 0.04	

[a]ORs adjusted for age at diagnosis, pigmentation and lifetime number of severe sunburns.
[b]ORs adjusted for age at diagnosis, pigmentation, lifetime number of severe sunburns and education level; includes only women who were asked about their OC use.

four or more severe sunburns (Table II). Interaction terms for sunburn history and *HAL* genotype were statistically significant (BCC: $P = 0.040$ and SCC: $P = 0.018$). There was no interaction between *HAL* genotype and pigment score (data not shown).

Modification by potential inducers of HAL transcription

As estrogen and glucocorticoids are potential transcriptional regulators of the *HAL* gene, we tested for gene–environment interaction with these exposures. Compared with those who never used oral glucocorticoids and were of the wild-type AA genotype, those who both had a variant and used oral glucocorticoids had a 2-fold increase in odds of BCC (OR = 2.3, 95% CI 0.9–5.6) and an almost 7-fold increase in odds of SCC (OR = 6.6, 95% CI 2.8–15.8) (Table III). The test for interaction between genotype and oral steroid use was statistically significant for SCC ($P < 0.01$) and borderline for BCC ($P < 0.06$). There was increased SCC risk associated with *HAL* genotype in women (OR = 1.5, 95% CI 1.1–2.2) (Table IV), but not in men (data not shown). Further, the elevation in risk associated with OC use was almost 4-fold (OR = 3.7, 95% CI 1.9–7.5) for those of the variant genotype, compared with wild-type non-users (Table IV). There was evidence of effect modification by genotype on OC usage in BCC (AG/GG: OR = 1.8, 95% CI 1.0–3.3), but this interaction was not statistically significant ($P = 0.11$).

Discussion

We analyzed the role of genetic variation in the histidase gene (*HAL*) in altering the risk of NMSCs and found evidence of effects in response to environmental and innate exposures, such as UV radiation, glucocorticoids and estrogen. Specifically, a high number of lifetime sunburns, indicative of intense UV exposure, increased risk of NMSCs in the presence of the variant G allele. Pigmentation did not modify the effects of *HAL* genotype on risk of skin cancer. Additional interactions were found with estrogen and glucocorticoids, both transcriptional activators of histidase expression. Glucocorticoid users had an increased risk of SCC in the presence of the variant G allele, as did women and those with a history of OC usage.

To our knowledge, the functionality of this SNP has not been studied previously in either humans or animal models. While the change from an isoleucine to a valine residue is relatively conservative, it is plausible that the amino acid change alters substrate affinity in the catalytic binding pocket. Nearby amino acid 444 is a highly conserved phenylalanine group present in humans, rats and mice, as well as plant, yeast and bacterial strains such as *Pseudomonas putida* (24). In experiments on histidase from *P.putida*, mutation of this phenylalanine results in a 100 to 2500-fold decrease in activity, as well as a 4-fold decrease in affinity for histidine (25,26). Based on the crystal structure, phenylalanine 444 is hypothesized to be involved in π-stacking interactions with a catalytic intermediate, resulting in stabilization of that intermediate to allow extraction of the appropriate β-proton (25). There is also evidence that loss of this residue results in

Downloaded from https://academic.oup.com/carcin/article/29/10/1950/2476412 by guest on 21 August 2022

improper formation of the 4-methylidene-imidazol-5-one group (25,27), the active site of this enzyme. In light of the importance of the positioning of this residue, an alteration in a neighboring amino acid such as I439V could potentiate a slight repositioning of F444, resulting in altered enzyme kinetics. The structure prediction software PMut (http://mmb2.pcb.ub.es:8080/PMut/) was used to estimate the effects this amino acid change would have on protein structure, based on sequence information. The program predicted this coding change to be neutral, suggesting that I439V may not be the causal SNP for the associations found in this study with NMSC. However, these results must be interpreted with caution, as the PMut software only has a 66.5% rate of improvement over random with sequence data (28). While this is one of the best programs currently available, the results are not definitive.

Whereas I439V is the only non-synonymous coding SNP, HapMap lists at least 40 synonymous coding or non-coding SNPs with minor allele frequencies > 0.05 in the 32.2 kb region (nucleotides 94864874–94897073) encoding for and surrounding *HAL*. It remains possible that I439V may not be directly causing the effect, but may instead be linked to another functional SNP in the untranslated region, promoter or intronic regions. The PMut prediction of a neutral substitution supports this theory. Also, the interactions we have found between genotype and two transcriptional inducers of *HAL* could be due to a linked promoter SNP as the causal variant.

In vitro studies have shown that increased exposure to UV directly correlates with increased UCA isomerization rates (29). As isomerization is a key on/off switch controlling production of *cis*-UCA, intense sun exposure could create higher epidermal concentration of *cis*-UCA and a consequent immunosuppressed state. Our results from the combined effects of *HAL* genotype and lifetime number of severe sunburns support this. If a high lifetime number of severe sunburns is considered a marker of intense UV exposure, our data show a gene dosage effect with increasing numbers of the variant G allele for both BCC and SCC among those with intense exposure. Therefore, our results suggest a role for intense, intermittent sun exposures in the etiology of UV-induced immunosuppression and NMSC.

The lack of biologic interaction between pigmentation and genotype agrees with previous literature that has shown a lack of effect of pigmentation on UV-induced immunosuppression. Vermeer *et al.* (10) found that UV-B susceptibility was a polymorphic trait that occurred at similar frequencies in Caucasian skin, heavily tanned skin and genetically determined black skin. This seems logical when considering the distribution of melanin among the layers of the epidermis. While melanin forms crescent-shaped vesicles over the nucleus of basal keratinocytes, they are degraded throughout the differentiation process, appearing as sparse particles in the uppermost stratum corneum (30). This degradation process most probably impairs the ability of melanin to effectively absorb UV in this top layer of the skin, allowing UCA photoisomerization to occur regardless of pigmentation.

Numerous studies in rats have shown a strong enhancement of hepatic histidase expression in the presence of estrogen (16–18), with postpubescent females possessing greatly increased levels of histidase throughout adulthood, compared with male counterparts. However, little research has been devoted to confirming estrogenic regulation in the skin. Estrogen receptors, specifically estrogen receptor-β, are expressed in human keratinocytes (31–33), and estrogen is known to promote proliferation of keratinocytes, wound healing and vascularization of skin (reviewed in ref. 34). However, the effects of estrogen on epidermal histidase have not been well characterized. Only one study has been performed on rat skin, showing decreased levels of histidase activity in adult females compared with adult males (35). However, epidermal thickness and stage of the hair follicle cycle were not accounted for in this study. As these correlate to the pattern of histidase activity, it is possible that one or both could be confounding the observed gender differences. Since there is a lack of validation of these findings in humans, the regulation of histidase by estrogen in the skin remains controversial. However, our findings lend support to the theory that human epidermal transcription of *HAL* may be regulated similarly to that of rat hepatic *HAL* transcription.

Similarly, studies in male rat livers show that glucocorticoids, specifically hydrocortisone acetate, are effective in elevating histidase levels (17). No studies performed in any model system have examined epidermal regulation of histidase by glucocorticoids. Therefore, studies into regulation of histidase by both estrogen and glucocorticoids in human keratinocytes are necessary to confirm the results found in rats and in our study.

While the effects of sunburns in combination with I439V seem to be similar between the two types of NMSC, differential effects on SCC and BCC appear to occur in the presence of estrogen or glucocorticoids and *HAL* genotype. This corroborates with other epidemiological data that transplant patients on immunosuppressive therapy are more likely to develop SCCs than BCCs (36,37), the latter of which are more prevalent in the general population. Additionally, OC usage has been shown in this case–control population to increase risk of SCC but not BCC (38). However, there is currently no known mechanism for these differences between the histologies.

A limitation of this study worth noting is that many variables used are self-reported. Therefore, the possibility of reporting bias, particularly with sun exposure variables, exists. One Australian study found little evidence of systematic changes in recall of severe sunburn history, when participants were surveyed repeatedly (39). Also, intraclass correlation coefficients did not significantly differ between cases and controls in this study. Additionally, for self-reporting of OC usage, Nischan *et al*. (40) showed in a breast cancer case–control population that there was no differential reporting of OC usage by case status. Finally, a previous study of this population showed that equal numbers of cases and controls reported using inhaled glucocorticoid steroids, implying that recall of oral steroid usage is probably also not related to case status (41).

NMSC has an estimated incidence of >1 million cases per year in the USA (42), and it is also one of the top five most costly cancers to Medicare, with treatment costs of over half a billion dollars in 1994–1995 (43). This cost was up 41% from 1992 to 1993 (43), and that number can be expected to grow as the ‘Baby Boomer’ generation reaches the peak age of onset. Additionally, ~11 million women in the USA are currently using OCs (44), and another 10 million new prescriptions for oral corticoid steroids for men and women are written each year to treat a variety of inflammatory diseases (reviewed in ref. 45). With such widely prevalent exposures and such a high morbidity rate of this disease, understanding the underlying susceptibilities and targeting at-risk populations early may help alleviate some of this burden. This highlights the importance of genetic susceptibility studies, such as this one, to understanding the etiology of NMSC and UV-induced immunosuppression. This research may have impact beyond even skin cancer as UV-induced immunosuppression has been associated with decreased pathogen response (46–50), interferes with vaccine efficacy (51) and has even been hypothesized to accelerate the onset and progression of AIDS in HIV-infected individuals (52). Given the potential scope of effects UV-induced immunosuppression has, further investigation of its genetic basis is crucial.

Funding

National Institute of Environmental Health Sciences (T32 ES007155); National Institutes of Health (R01CA082354 and R01CA57494).

Acknowledgements

Conflict of Interest Statement: None declared.

References

1. Prater,M.R. (2003) Immunotoxic effects of cis-urocanic acid exposure in C57BL/6N and C3H/HeN mice. *Photochem. Photobiol.*, **77**, 383–389.
2. Hart,P.H. (1999) A critical role for dermal mast cells in cis-urocanic acid-induced systemic suppression of contact hypersensitivity responses in mice. *Photochem. Photobiol.*, **70**, 807–812.

Downloaded from https://academic.oup.com/carcin/article/29/10/1950/2476412 by guest on 21 August 2022

3. Hart,P.H. (1997) Histamine involvement in UVB- and cis-urocanic acid-induced systemic suppression of contact hypersensitivity responses. *Immunology*, **91**, 601–608.
4. Beissert,S. (2001) IL-12 prevents the inhibitory effects of cis-urocanic acid on tumor antigen presentation by Langerhans cells: implications for photocarcinogenesis. *J. Immunol.*, **167**, 6232–6238.
5. Beissert,S. (1997) Regulation of tumor antigen presentation by urocanic acid. *J. Immunol.*, **159**, 92–96.
6. Khalil,Z. (2001) cis-Urocanic acid stimulates neuropeptide release from peripheral sensory nerves. *J. Invest. Dermatol.*, **117**, 886–891.
7. Moodycliffe,A.M. (1996) Differential effects of a monoclonal antibody to cis-urocanic acid on the suppression of delayed and contact hypersensitivity following ultraviolet irradiation. *J. Immunol.*, **157**, 2891–2899.
8. Noonan,F.P. *et al*. (1994) Susceptibility to immunosuppression by ultraviolet B radiation in the mouse. *Immunogenetics*, **39**, 29–39.
9. Yoshikawa,T. (1990) Susceptibility to effects of UVB radiation on induction of contact hypersensitivity as a risk factor for skin cancer in humans. *J. Invest. Dermatol.*, **95**, 530–536.
10. Vermeer,M. (1991) Effects of ultraviolet B light on cutaneous immune responses of humans with deeply pigmented skin. *J. Invest. Dermatol.*, **97**, 729–734.
11. Streilein,J.W. *et al*. (1988) Genetic basis of ultraviolet-B effects on contact hypersensitivity. *Immunogenetics*, **27**, 252–258.
12. Hanford,W.C. *et al*. (1977) Genetic differences in the rate of histidase synthesis in inbred mice. *J. Biol. Chem.*, **252**, 6695–6699.
13. Yoshikawa,T. *et al*. (1990) Genetic basis of the effects of ultraviolet light B on cutaneous immunity. Evidence that polymorphism at the Tnfa and Lps loci governs susceptibility. *Immunogenetics*, **32**, 398–405.
14. Suchi,M. (1995) Molecular cloning and structural characterization of the human histidase gene (HAL). *Genomics*, **29**, 98–104.
15. Hryb,D.J. *et al*. (1983) Nature of translational products, tissue specificity, and differential development in male and female rat liver. *J. Biol. Chem.*, **258**, 11377–11383.
16. Feigelson,M. (1968) Estrogenic regulation of hepatic histidase during postnatal development and adulthood. *J. Biol. Chem.*, **243**, 5088–5093.
17. Feigelson,M. (1973) Studies on the postnatal development of responses of hepatic histidase to endocrine control. *Biochim. Biophys. Acta.*, **304**, 669–685.
18. Bhargava,M.M. *et al*. (1976) Studies on the mechanisms of histidase development in rat skin and liver. II. Alterations in enzyme levels and synthetic rates during development. *Dev. Biol.*, **48**, 226–236.
19. Karagas,M.R. (1999) Increase in incidence rates of basal cell and squamous cell skin cancer in New Hampshire, USA. New Hampshire Skin Cancer Study Group. *Int. J. Cancer*, **81**, 555–559.
20. Karagas,M.R. (2001) Skin cancer risk in relation to toenail arsenic concentrations in a US population-based case-control study. *Am. J. Epidemiol.*, **153**, 559–565.
21. Miettinen,O.S. (1976) Stratification by a multivariate confounder score. *Am. J. Epidemiol.*, **104**, 609–620.
22. Cook,E.F. *et al*. (1989) Performance of tests of significance based on stratification by a multivariate confounder score or by a propensity score. *J. Clin. Epidemiol.*, **42**, 317–324.
23. Miller,K.L. (2006) XPA, haplotypes, and risk of basal and squamous cell carcinoma. *Carcinogenesis*, **27**, 1670–1675.
24. Taylor,R.G. *et al*. (1994) Site-directed mutagenesis of conserved serines in rat histidase. Identification of serine 254 as an essential active site residue. *J. Biol. Chem.*, **269**, 27473–27477.
25. Rother,D. (2001) Characterization of the active site of histidine ammonia-lyase from *Pseudomonas putida*. *Eur. J. Biochem.*, **268**, 6011–6019.
26. Baedeker,M. *et al*. (2002) Structures of two histidine ammonia-lyase modifications and implications for the catalytic mechanism. *Eur. J. Biochem.*, **269**, 1790–1797.
27. Baedeker,M. *et al*. (2002) Autocatalytic peptide cyclization during chain folding of histidine ammonia-lyase. *Structure*, **10**, 61–67.
28. Ferrer-Costa,C. *et al*. (2004) Sequence-based prediction of pathological mutations. *Proteins*, **57**, 811–819.
29. Morrison,H. (1985) Photochemistry and photobiology of urocanic acid. *Photodermatol.*, **2**, 158–165.
30. Standring,S. (ed.) 2004 *Gray's Anatomy: The Anatomical Basis of Clinical Practice*, 39th edn. C.V. Mosby, New York, NY, 39th edn1627.
31. Thornton,M.J. (2003) The distribution of estrogen receptor beta is distinct to that of estrogen receptor alpha and the androgen receptor in human skin and the pilosebaceous unit. *J. Investig. Dermatol. Symp. Proc.*, **8**, 100–103.
32. Pelletier,G. *et al*. (2004) Localization of sex steroid receptors in human skin. *Histol. Histopathol.*, **19**, 629–636.
33. Thornton,M.J. (2003) Oestrogen receptor beta is the predominant oestrogen receptor in human scalp skin. *Exp Dermatol.*, **12**, 181–190.
34. Thornton,M.J. (2002) The biological actions of estrogens on skin. *Exp. Dermatol.*, **11**, 487–502.
35. Reaven,E.P. *et al*. (1967) The relationship of histochemically reactive histidine to histidase activity in the epidermis of growing rats. *Anat. Rec.*, **158**, 139–147.
36. Ulrich,C. (2004) Comparative epidemiology and pathogenic factors for nonmelanoma skin cancer in organ transplant patients. *Dermatol. Surg.*, **30**, 622–627(4 Pt 2).
37. McCann,J. (1999) Can skin cancers be minimized or prevented in organ transplant patients? *J. Natl Cancer Inst.*, **91**, 911–913.
38. Miller-Applebaum,K. *et al*. (2005) Oral contraceptives and non-melanoma skin cancer. *Environmental, Hormonal, and Genetic Risk Factors for Non-melanoma Skin Cancer* [*dissertation*]. Harvard School of Public Health, Boston, MA.
39. English,D.R. *et al*. (1998) Reproducibility of reported measurements of sun exposure in a case-control study. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 857–863.
40. Nischan,P. (1993) Comparison of recalled and validated oral contraceptive histories. *Am. J. Epidemiol.*, **138**, 697–703.
41. Karagas,M.R. (2001) Non-melanoma skin cancers and glucocorticoid therapy. *Br. J. Cancer*, **85**, 683–686.
42. American Cancer Society. *Cancer Facts and Figures 2007*. American Cancer Society, Atlanta.
43. Housman,T.S. (2003) Skin cancer is among the most costly of all cancers to treat for the Medicare population. *J. Am. Acad. Dermatol.*, **48**, 425–429.
44. Mosher,W.D. (2004) Use of contraception and use of family planning services in the United States: 1982–2002. *Adv. Data*, 1–36.
45. Schacke,H. *et al*. (2002) Mechanisms involved in the side effects of glucocorticoids. *Pharmacol. Ther.*, **96**, 23–43.
46. Denkins,Y. *et al*. (1989) Exposure of mice to UV-B radiation suppresses delayed hypersensitivity to *Candida albicans*. *Photochem. Photobiol.*, **49**, 615–619.
47. Jeevan,A. *et al*. (1989) Effect of a single exposure to ultraviolet radiation on *Mycobacterium bovis* bacillus Calmette-Guerin infection in mice. *J. Immunol.*, **143**, 2837–2843.
48. Giannini,M.S. (1986) Suppression of pathogenesis in cutaneous leishmaniasis by UV irradiation. *Infect. Immun.*, **51**, 838–843.
49. Howie,S. *et al*. (1986) Exposure to low-dose ultraviolet radiation suppresses delayed-type hypersensitivity to herpes simplex virus in mice. *J. Invest. Dermatol.*, **86**, 125–128.
50. Ross,J.A. (1986) Ultraviolet-irradiated urocanic acid suppresses delayed-type hypersensitivity to herpes simplex virus in mice. *J. Invest. Dermatol.*, **87**, 630–633.
51. Sleijffers,A. (2003) Epidermal cis-urocanic acid levels correlate with lower specific cellular immune responses after hepatitis B vaccination of ultraviolet B-exposed humans. *Photochem. Photobiol.*, **77**, 271–275.
52. Vincek,V. (1995) Sunlight induced progression of AIDS. *Med. Hypotheses*, **44**, 119–123.

Received April 2, 2008; revised June 27, 2008; accepted July 2, 2008

Downloaded from https://academic.oup.com/carcin/article/29/10/1950/2476412 by guest on 21 August 2022