Staphylococcus aureus and Squamous Cell Carcinoma of the Skin

Johanna Kullander, Ola Forslund, and Joakim Dillner

Department of Laboratory Medicine, Division of Medical Microbiology, Lund University, University Hospital, Malmö, Sweden

Abstract

Squamous cell carcinoma (SCC) of the skin is a tumor with greatly increased incidence among immunosuppressed patients; therefore, an infectious cause of SCC has long been sought. We performed a hospital-based case-control study of *Staphylococcus aureus* and biopsies of SCC (n = 82), basal cell carcinoma (n = 142), actinic keratosis (n = 57), and seborrhoeic keratosis (n = 72) in comparison with biopsies from healthy skin of these 353 immunocompetent patients. In a *S. aureus*-specific PCR, targeting the *nuc* gene, presence of *S. aureus* DNA was strongly associated with SCC (29.3% positive specimens; adjusted odds ratio, 6.23; 95% confidence interval, 3.10-12.53) compared with healthy skin (5.7% positive specimens). There was also a tendency for association of *S. aureus* with actinic keratosis, but no association was found for basal cell carcinoma or seborrhoeic keratosis. Analysis using cotton swab samples taken on top of the lesions and from healthy skin gave similar results (adjusted odds ratio for SCC compared with healthy skin, 2.67; 95% confidence interval, 1.47-4.83). In conclusion, there is a strong association between SCC and presence of *S. aureus*. The study design used cannot determine whether the association implies that presence of *S. aureus* might influence carcinogenesis or whether it may imply that SCC has an increased susceptibility to *S. aureus* colonization. (Cancer Epidemiol Biomarkers Prev 2009;18(2):472–8)

Introduction

Nonmelanoma skin cancer, including squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), is the most common cancer among Caucasians, and there has been an increase in prevalence in Europe during the last few years (1, 2). The main risk factor for developing nonmelanoma skin cancer is exposure to ultraviolet radiation (3). SCC has a greatly increased incidence among the immunosuppressed, 65- to 250-fold in organ recipients (4-6). Among these patients, the impaired immunosurveillance against viral antigens results in increased development of skin warts as well as several virus-associated cancers such as Kaposi's sarcoma, EBV-associated lymphoma, and human papillomavirus (HPV)-associated cervical cancer (7-9), implying that the immunosuppression specifically induces an impaired ability to control tumorigenic viruses. Therefore, an infectious cause of SCC has long been sought. An association of nonmelanoma skin cancer with HPV infection was first shown in patients with the rare disease epidermodysplasia verruciformis (10). These patients have a high risk of developing HPV-positive nonmelanoma skin cancer. Cutaneous HPV is commonly found among the immunocompetent both in benign and malignant skin lesions (11) and on healthy skin (12). In case-control studies, presence of HPV of the β2 species has been associated with SCC (13, 14). However, these studies provided no information on the direction of causality of the association and the associations have also not been particularly strong.

More than 15% of malignancies worldwide are established to have an infectious cause (15). The main mechanisms by which infections can cause cancer are (*a*) transformation of cells by insertion of oncogenes and/or inhibition of tumor suppressors (e.g., oncogenic HPV types, the major cause of cervical cancer, interact with the tumor suppressor protein p53 and target it for degradation; ref. 16); (*b*) carcinogenesis induced by immunosuppression (e.g., in HIV-related cancers; ref. 17), and (*c*) infection-induced chronic inflammation can produce nitric oxide and cytokines, which contribute to carcinogenesis (18, 19) (e.g., *Helicobacter pylori*, the first bacterium to be identified as a definite cause of cancer in humans by the IARC (20) causes a chronic inflammation in the gastric mucosa, which is likely to be responsible for the development of cancer; ref. 21).

The present study started as an investigation of whether SCC may contain as yet unidentified HPV types or other microorganisms. We performed an unbiased molecular isolation of microorganisms from biopsies of SCC using multiple displacement amplification (MDA) with random primers (22). As several of the detected sequences were different plasmids from *Staphylococcus aureus*, we decided to investigate whether *S. aureus* was more common in SCC than in other skin lesions or in healthy skin using a formal hospital-based case-control study. Following development of a *S. aureus*-specific PCR targeting the *nuc* gene, we surveyed a series of >350 biopsies and swab samples from skin lesions with paired controls from healthy skin.

Patients and Methods

Patients and Sample Collection. The study was designed as a hospital-based case-control study of

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Requests for reprints: Joakim Dillner, Department of Laboratory Medicine, Division of Medical Microbiology, Lund University, University Hospital, Malmö S-205 02, Sweden. Phone: 46-40-338126; Fax: 46-40-337312. E-mail: joakim.dillner@med.lu.se

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nonmelanoma skin cancers, premalignant and benign lesions (13). Patients included in this study are those who sought medical care at participating dermatology clinics, where surgical removal of a skin lesion was medically indicated and the patients gave informed consent to participate. Skin biopsies were collected from 358 patients with no known immunosuppression, attending Swedish (Stockholm, 126 patients; Gothenburg, 72 patients; and Malmö, 114 patients) and Austrian (Vienna, 46 patients) hospitals. The sample series included SCC (n = 87; mean age, 79 years; range, 42-94 years), BCC (n = 142; mean age, 75 years; range, 38-97 years), actinic keratosis (n = 57; mean age, 77 years; range, 59-95 years), and seborrhoeic keratosis (n = 72; mean age, 73 years; range, 43-97 years). From each patient, samples were collected from the top of the lesions by a pre-wetted (0.9% NaCl) cotton-tipped swab that was rolled on the lesion (within margins of the lesion) and from healthy adjacent skin where the cotton-tipped swabs were drawn to an fro 15 times within an area of 5 × 5 cm and suspended in 1 mL saline. After tape stripping (23), a biopsy was taken from the lesion and from adjacent (10-15 cm from the lesion) healthy skin of the same patient. The DNA was extracted using a phenol-free method (24). Five patients with SCC were excluded: 2 because of immunosuppression and 3 because of inadequate amounts of sample. Hence, 1,412 samples were included in the study. All patients provided informed consent and the studies were approved by the appropriate ethical review boards in Sweden and Austria.

Patients were interviewed by use of a standardized questionnaire inquiring about skin type [I-IV, according to the Fitzpatrick classification (25)], history of sunburns, eye color, and smoking habits. The level of sun exposure at the site of biopsy was classified by a single dermatologist (Dr. Bernt Lindelöf, Karolinska Institute) as being in one of three categories based on anatomic sites: extensive (head, neck and dorsal side of the hands), moderate (trunk and extremities), or low (buttock and the genital area). To avoid visible scars, the control biopsy was not always taken at an equally sun exposed area as the biopsy of the lesion.

HPV positivity was investigated in a previous study (13).

MDA and Cloning. To preferentially amplify HPV, MDA with random and HPV-generic primers was done on 83 SCC biopsies (26). After digestion with 5 units *Hin*cII (New England Biolabs), the samples were run on a 1% agarose gel. By visualization, strong, separate bands were excised, purified with QIAquick gel extraction kit (Qiagen), and cloned with Zero Blunt TOPO PCR Cloning Kit (Invitrogen). Sequencing used primer walking with ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction kits (Applied Biosystems) and a 3730 sequencer (Applied Biosystems).

To increase the amount of DNA from biopsies from the lesion and the adjacent healthy skin, MDA was also done with random hexamers. Extracted DNA (1 μL) and 3′-thiophosphate-5′-nitroindole-modified random hexamer (220 μmol/L; Fidelity Systems), 44 mmol/L Tris-HCl (pH 8), and 11 mmol/L $MgCl_2$ (Roche) in a volume of 4.5 μL were denatured for 3 min at 94°C, cooled on ice, and mixed with a 14.5 μL solution containing 10 units Phi29 polymerase (Fermentas), 1× Phi29 polymerase reaction buffer (Fermentas), and 1.4 mmol/L deoxynucleotriphosphate (Roche). Incubation for 16 h at 30°C, inactivation for 10 min at 65°C, dilution 1:2 in TE buffer [10 mmol/L Tris (pH 8), 1 mmol/L EDTA], and shaking at 500 rpm at 4°C for 8 h followed.

Analysis of *S. aureus* DNA. *S. aureus* was detected by PCR amplification of a ~270-bp region in the *nuc* gene (27). The 25 μL reaction mix contained 50 μmol/L of each primer (DNA Technology), 1× Buffer II (Applied Biosystems), 0.35 mmol/L $MgCl_2$ (Applied Biosystems), 0.2 mmol/L of each deoxynucleotriphosphate (Fermentas), 0.2% bovine serum albumin (Sigma-Aldrich), 0.625 units AmpliTaq Gold (Applied Biosystems), and 2.5 μL sample. The PCR was carried out in an Eppendorf Mastercycler with the following variables: an initial denaturation step of 10 min at 94°C followed by 45 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. Samples were analyzed with SYBR Green. The amplified material (9 μL) was mixed with 1 μL of 10× SYBR Green (Invitrogen) and a dissociation curve with 60°C as start temperature was carried out in a GeneAmp 5700 SDS (Applied Biosystems). Dilutions from 20,000 to 2 colony-forming units *S. aureus* per reaction were used as positive control with the lowest dilution positive every time. All samples containing detectable product of the same melting point as *S. aureus* (83.5°C) were confirmed by gel electrophoresis. All samples were also analyzed for human DNA content using real-time PCR for the *β-globin* gene (28). To prevent PCR contamination, the samples were prepared in a room separated from the PCR.

Statistical Analysis. LogXact (version 8; Cytel Software) was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) by use of multivariate logistic regression. If models did not converge, the models were analyzed in the statistical software R version 2.7.2 (29) and remedied using ''model simplification'' excluding variables using single term deletions as recommended in the software.

Results

A total of 22 of 83 (27%) samples showed strong bands by gel electrophoresis after MDA and treatment with *Hin*cII. From these 22 patients, 28 bands were cloned and sequenced and 12 (43%) of these sequences were found to be *S. aureus*. Fourteen bands contained sequences from the human genome. The last two were *Escherichia coli* and a new HPV type, later designated HPV109 (data not shown).

The prevalence of *S. aureus* in biopsies was highest among SCC lesions (Table 1A). Overall, 5.7% of healthy skin biopsies were positive compared with 1.4% of seborrhoeic keratosis, 12.3% of actinic keratosis, 7.7% of BCC, and 29.3% of SCC biopsies (Table 1A). The prevalence of *S. aureus* in swab samples from adjacent healthy skin was 15.0%, 19.4% in samples taken on top of the lesion from seborrhoeic keratosis, 26.3% from actinic keratosis, 13.4% from BCC, and 31.7% from SCC (Table 1B).

In the primary analysis with biopsies from healthy skin as the reference and after adjustment for age, sex,

Table 1. Risk factors for *S. aureus* DNA in (A) tumor biopsies and (B) swab samples with healthy skin as reference

(A)

Category	*S. aureus* status, *n* (%) samples*		OR (95% CI)	Adjusted OR† (95% CI)
	Positive biopsy (*n* = 63)	Negative biopsy (*n* = 643)		
Diagnosis				
Healthy skin	20 (5.7)	333 (94.3)	1.0 (reference)	1.0 (reference)
Seborrhoeic keratosis	1 (1.4)	71 (98.6)	0.24 (0.0056-1.52)	0.26 (0.034-2.0)
Actinic keratosis	7 (12.3)	50 (87.7)	2.33 (0.79-6.10)	2.18 (0.83-5.75)
BCC	11 (7.7)	131 (92.3)	1.40 (0.59-3.16)	1.41 (0.65-3.08)
SCC	24 (29.3)	58 (70.7)	6.84 (3.38-14.01)‡	6.23 (3.10-12.53)‡
Age (y)				
≤60	3 (6.8)	41 (93.2)	1.0 (reference)	1.0 (reference)
61-75	16 (6.1)	248 (93.9)	0.88 (0.24-4.93)	0.86 (0.21-3.49)
≥76	44 (11.1)	354 (88.9)	1.70 (0.51-8.92)	1.26 (0.33-4.86)
Sex				
Female	24 (8.2)	270 (91.8)	1.0 (reference)	1.0 (reference)
Male	39 (9.5)	373 (90.5)	1.18 (0.67-2.10)	1.19 (0.64-2.21)
Skin type				
I	5 (6.8)	69 (93.4)	1.0 (reference)	1.0 (reference)
II	17 (9.9)	154 (90.1)	1.52 (0.51-5.49)	1.66 (0.55-5.00)
III	34 (8.9)	349 (91.1)	1.34 (0.50-4.56)	1.27 (0.44-3.62)
IV	7 (9.5)	67 (90.5)	1.44 (0.37-6.05)	0.99 (0.26-3.75)
Previous sunburn				
Never	14 (14.3)	84 (85.7)	1.0 (reference)	1.0 (reference)
A few times	27 (8.9)	277 (91.1)	0.59 (0.28-1.27)	0.57 (0.26-1.29)
Sometimes	20 (8.4)	218 (91.6)	0.55 (0.25-1.24)	0.51 (0.22-1.21)
Every year	2 (3.0)	64 (97.0)	0.19 (0.020-0.87)‡	0.20 (0.038-0.99)‡
Eye color				
Blue/grey	51 (9.7)	475 (90.3)	1.0 (reference)	1.0 (reference)
Green/mixed	5 (4.5)	107 (95.5)	0.44 (0.13-1.12)	0.48 (0.18-1.31)
Brown	7 (10.3)	61 (89.7)	1.07 (0.39-2.51)	1.19 (0.49-2.91)
Sun exposure				
Low/moderate	29 (6.9)	394 (93.1)	1.0 (reference)	1.0 (reference)
Extensive	34 (12.1)	248 (87.9)	1.86 (1.07-3.25)‡	1.25 (0.67-2.32)
Smoking				
Never smoked	28 (8.4)	306 (91.6)	1.0 (reference)	1.0 (reference)
Smoked before, but stopped	27 (8.9)	275 (91.1)	1.073 (0.59-1.94)	1.10 (0.58-2.10)
Yes	8 (11.4)	62 (88.6)	1.41 (0.53-3.37)	1.79 (0.71-4.52)
HPV-positive biopsy				
Negative	41 (8.2)	457 (91.8)	1.0 (reference)	1.0 (reference)
HPV-positive, non-β2	10 (7.4)	126 (92.6)	0.88 (0.38-1.86)	0.81 (0.37-1.77)
HPV-positive, β2	12 (16.7)	60 (83.3)	2.23 (1.01-4.62)‡	1.52 (0.68-3.41)

(B)

Category	*S. aureus* status, *n* (%) samples*		OR	Adjusted OR† (95% CI)
	Positive swab sample (*n* = 127)	Negative swab sample (*n* = 579)		
Diagnosis				
Healthy skin	53 (15.0)	300 (85.0)	1.0 (reference)	1.0 (reference)
Seborrhoeic keratosis	14 (19.4)	58 (80.6)	1.37 (0.66-2.70)	1.72 (0.87-3.39)
Actinic keratosis	15 (26.3)	42 (73.7)	2.02 (0.97-4.04)	1.75 (0.87-3.54)
BCC	19 (13.4)	123 (86.6)	0.87 (0.47-1.58)	0.88 (0.49-1.58)
SCC	26 (31.7)	56 (68.3)	2.62 (1.45-4.69)‡	2.67 (1.47-4.83)‡
Age (y)				
≤60	6 (13.6)	38 (86.4)	1.0 (reference)	1.0 (reference)
61-75	35 (13.3)	229 (86.7)	0.97 (0.37-3.01)	0.82 (0.31-2.20)
≥76	86 (21.6)	312 (78.4)	1.74 (0.70-5.22)	1.36 (0.52-3.54)
Sex				
Female	59 (20.1)	235 (79.9)	1.0 (reference)	1.0 (reference)
Male	68 (16.5)	344 (83.5)	0.79 (0.53-1.18)	0.78 (0.50-1.21)
Skin type				
I	17 (23.0)	57 (77.0)	1.0 (reference)	1.0 (reference)
II	36 (21.0)	135 (79.0)	0.89 (0.45-1.84)	0.88 (0.44-1.76)
III	59 (15.4)	324 (84.6)	0.61 (0.32-1.20)	0.55 (0.29-1.07)
IV	14 (18.9)	60 (81.1)	0.78 (0.32-1.87)	0.64 (0.26-1.55)

(*Continued on the following page*)

Table 1. Risk factors for *S. aureus* DNA in (A) tumor biopsies and (B) swab samples with healthy skin as reference (Cont'd)

(B)

Category	*S. aureus* status, *n* (%) samples*		OR	Adjusted OR† (95% CI)
	Positive swab sample (*n* = 127)	Negative swab sample (*n* = 579)		
Previous sunburn				
Never	22 (22.5)	76 (77.5)	1.0 (reference)	1.0 (reference)
A few times	55 (18.1)	249 (81.9)	0.76 (0.43-1.40)	0.94 (0.50-1.78)
Sometimes	41 (17.2)	197 (82.8)	0.72 (0.39-1.36)	0.84 (0.43-1.64)
Every year	9 (13.6)	57 (86.4)	0.55 (0.21-1.35)	0.58 (0.22-1.51)
Eye color				
Blue/grey	98 (18.6)	428 (81.4)	1.0 (reference)	1.0 (reference)
Green/mixed	12 (10.7)	100 (89.3)	0.52 (0.25-1.01)	0.45 (0.23-0.91)‡
Brown	17 (25)	51 (75)	1.46 (0.75-2.69)	1.64 (0.88-3.07)
Sun exposure				
Low/moderate	66 (15.6)	357 (84.4)	1.0 (reference)	1.0 (reference)
Extensive	61 (21.6)	221 (78.4)	1.49 (0.99-2.24)	1.24 (0.80-1.92)
Smoking				
Never smoked	64 (19.2)	270 (80.8)	1.0 (reference)	1.0 (reference)
Smoked before, but stopped	55 (18.2)	247 (81.8)	0.94 (0.62-1.43)	1.17 (0.75-1.84)
Yes	8 (11.4)	62 (88.6)	0.55 (0.21-1.22)	0.74 (0.32-1.71)
HPV positivity				
Negative	60 (16.4)	306 (83.6)	1.0 (reference)	1.0 (reference)
Positive	67 (19.8)	271 (80.2)	1.26 (0.84-1.89)	1.35 (0.88-2-05)

*Because some subjects did not provide all information, the sum of the total numbers in the subgroups may be less than the total number of subjects.
† Adusted for all variables.
‡ Significant associations.

skin type, previous sunburn, eye color, sun exposure at biopsy site, smoking, and HPV positivity in the biopsy, presence of *S. aureus* DNA in skin biopsies was strongly associated with a SCC diagnosis (OR, 6.23; 95% CI, 3.10-12.53; Table 1A). *S. aureus* DNA in swab samples was also strongly associated with SCC lesions (OR, 2.67; 95% CI, 1.47-4.83; Table 1B) with swabs from healthy skin as the reference and after adjustment for age, sex, skin type, previous sunburn, eye color, sun exposure at swab site, smoking, and HPV positivity in swab (13).

To investigate whether the association of SCC and *S. aureus* might be explained by tendency to adhere to any protruding growth of the skin, we performed an alternative analysis with the benign protruding growth seborrhoeic keratosis as the reference. In this analysis, there was an even stronger association of *S. aureus* DNA and SCC biopsies (OR, 23.84; 95% CI, 3.69-1,004) and also a weak association for actinic keratosis (OR, 10.01; 95% CI, 1.37-∞; Table 2A).

To investigate whether the association could be explained by SCC subjects being generally more susceptible to *S. aureus*, we compared the lesions with healthy skin biopsies from the same patient. *S. aureus* DNA was more frequently detected in SCC (OR, 7.26; 95% CI, 1.38-76.55) than in the matched healthy skin samples from the same subject (Table 3A).

In only 0.8% (3 of 353) study subjects, all SCC patients, was *S. aureus* DNA found in all four samples (biopsies from lesion and healthy skin and swabs from top of lesion and healthy skin).

S. aureus in swab samples appeared to associate negatively with green or mixed eye color (OR, 0.45; 95% CI, 0.23-0.91); also, a history of sunburn every year was negatively associated with *S. aureus* DNA in biopsies (adjusted OR, 0.20; 95% CI, 0.038-0.99; Table 1A and B).

Discussion

In this study, we report a strong association of *S. aureus* DNA with SCC of the skin. The association was not merely restricted to the skin surface, as it was found in both biopsies and swab samples taken on top of the lesions. As the association was at least as strong when using a benign exophytic growth (seborrhoeic keratosis) as reference, it appears that acquisition of bacteria by mere protrusion of the lesions from the skin surface cannot explain the association. Also, as *S. aureus* was more common in SCC biopsies than in healthy skin samples from the same patient, the association cannot be explained by genetic or disease-induced overall susceptibility to bacterial colonization.

S. aureus is a gram-positive coccus that may occur as a commensal on human skin and in the nose. Approximately 10% to 40% of adults are reported to be carriers (30, 31) as was also found in our study (15% of swab samples taken on healthy skin being positive). *S. aureus* is normally not able to infect an immunocompetent person unless normal barriers have been broken by, for example, surgery or burns (32), suggesting that the association of *S. aureus* with SCC might be due to the ulcerating growth characteristics of SCC that could promote *S. aureus* colonization. The prevalence of *S. aureus* in biopsies from skin lesions varied from 1.4% in seborrhoeic keratosis to 29.3% in SCC. To our knowledge, this study is the first to present prevalence data of *S. aureus* in biopsies from skin lesions. As we stripped the surface of the lesions with tape before taking of biopsies, surface contamination with microorganisms in the environment or other sites in the body is less likely.

It would be of interest to investigate whether the increased presence of *S. aureus* in SCC of the skin is due to the susceptibility to *S. aureus* colonization or if the

Table 2. Diagnosis associated with *S. aureus* DNA in (A) tumor biopsies and (B) swab samples when seborrhoeic keratosis is used as the reference

(A)

Diagnosis	Tumor biopsy, OR (95% CI)	Tumor biopsy, adjusted OR (95% CI)	Biopsy from healthy skin, OR (95% CI)	Biopsy from healthy skin, adjusted OR (95% CI)
Seborrhoeic keratosis	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Actinic keratosis	9.79 (1.20-454)*	10.01 (1.37-∞)*,†	0.84 (0.068-7.58)	1 (0.0037-273)†
BCC	5.93 (0.83-260)	2.45 (0.31-110)†	1.02 (0.21-6.46)	0.66 (0.077-5.78)†
SCC	28.93 (4.45-1,225)*	23.84 (3.69-1,004)*,‡	2.82 (0.67-16.85)	2.03 (0.36-14.78)†

(B)

Diagnosis	Swab samples from top of lesion, OR (95% CI)	Swab samples from top of lesion, adjusted OR (95% CI)	Swab samples from healthy skin, OR (95% CI)	Swab samples from healthy skin, adjusted OR (95% CI)
Seborrhoeic keratosis	1.0 (reference)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Actinic keratosis	1.48 (0.59-3.70)	1.32 (0.52-3.37)‡	1.32 (0.45-3.85)	1.26 (0.42-3.74)‡
BCC	0.64 (0.28-1.49)	0.66 (0.28-1.55)‡	0.79 (0.31-2.06)	0.81 (0.31-2.22)‡
SCC	1.92 (0.86-4.40)	1.80 (0.79-4.23)‡	2.82 (0.67-16.85)	1.61 (0.64-4.28)‡

*Significant associations.
† Adusted for all variables in Table 1.
‡ Adjusted for eye color, smoking, and HPV positivity in biopsy.

Table 3. Pairwise comparisons of *S. aureus* DNA in lesions with (A) paired healthy skin biopsies and (B) paired swab samples

(A)

Diagnosis	Tumor biopsy, *S. aureus* positivity, *n* (%)	Healthy skin biopsy, *S. aureus* positivity, *n* (%)	OR (95% CI)	Adjusted OR (95% CI)*
Seborrhoeic keratosis	1 (1.4)	3 (4.2)	0.33 (0.0064-4.15)	—†
Actinic keratosis	7 (12.3)	2 (3.5)	3.5 (0.67-34.53)	5.29 (0.66-∞)
BCC	11 (7.7)	6 (4.2)	1.83 (0.62-6.04)	3.33 (0.86-18.85)
SCC	24 (29.3)	9 (11.0)	4.75 (1.58-19.2)‡	7.26 (1.38-76.55)‡

(B)

Diagnosis	Swab sample on top of lesion, *S. aureus* positivity, *n* (%)	Healthy skin swab sample, *S. aureus* positivity, *n* (%)	OR (95% CI)	Adjusted OR (95% CI)*
Seborrhoeic keratosis	14 (19.4)	10 (13.9)	2.0 (0.54-9.08)	1.75 (0.44-8.15)
Actinic keratosis	15 (26.3)	10 (17.5)	6.0 (0.73-276)	2.85 (0.39-118)
BCC	19 (13.4)	16 (11.3)	1.5 (0.48-5.12)	1.73 (0.55-7.11)
SCC	26 (31.7)	17 (20.7)	5.5 (1.2-51.06)‡	5.56 (0.93-∞)

*Adjusted for sun exposure at biopsy site and HPV positivity. Because the lesion samples and healthy skin samples were taken from the same subjects, all other covariates are identical and therefore not included in the model.
† Too few positive biopsies to calculate adjusted OR.
‡ Significant associations.

bacterium could be causally involved in the development of cancer. A possible mechanism whereby *S. aureus* might contribute to tumor formation is by the production of a chronic inflammation, a classic mechanism of carcinogenesis (33). Chronic inflammation produces several different cytokines (19), for example, tumor necrosis factor (a major mediator of inflammation), which has been linked to all steps in tumor development. Tumor necrosis factor has also been suggested to activate nuclear factor-κB (34), a known tumor promoter in the development of hepatocellular carcinoma. The staphylococcal α-toxin has been shown to activate both several cytokines and nuclear factor-κB (35).

The increased prevalence of *S. aureus* DNA also in actinic keratosis biopsies (when compared with seborrhoeic keratosis) is of interest as actinic keratosis is considered a precursor to the development of SCC (36, 37), which could suggest that some increased colonization with *S. aureus* is seen already early on in the carcinogenic process.

We did not find any determinants of *S. aureus* positivity that would suggest that the association could be attributable to confounding, not fully compensated for in the multivariate analysis. On the contrary, patients with a history of sunburn every year (a known risk factor for SCC) had a decreased prevalence of *S. aureus* in their lesions. This finding was of borderline significance and could be a chance finding, although conceivable explanations exist (e.g., healthy lifestyle associating with less microorganisms).

The MDA reaction using random hexamers can amplify any DNA present in the sample, but if an infection is present at high copy number it will be preferentially amplified. HPV is known to be very commonly present in SCC but usually in very low amounts of virus (38-40). This is in line with our findings that MDA commonly amplifies *S. aureus* but only occasionally (a single tumor) amplifies HPV in SCC specimens.

In conclusion, we found a strong association of *S. aureus* DNA with SCC of the skin. The association is stronger than what has been found for SCC and HPV. Further studies on infections in the etiology of SCC should consider whether SCC causes staphylococcal colonization or vice versa as well as of general susceptibility to infections.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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