Evidence for the Association of Human Papillomavirus Infection and Cutaneous Squamous Cell Carcinoma in Immunocompetent Individuals

Cinzia Masini, MD; Pawel G. Fuchs, PhD†; Fabrizio Gabrielli, MD; Sabine Stark, DBiol; Francesco Sera, DStat; Miriam Ploner; Carmelo Franco Melchi, MD; Grazia Primavera, MD; Giulio Pirchio, MD; Orietta Picconi, DStat; Pierpaolo Petasecca, MD; Maria Sofia Cattaruzza, MD, MSc; Herbert J. Pfister, PhD; Damiano Abeni, MD, MPH

Objective: The aim of our study was to evaluate human papillomavirus (HPV) infection as a risk factor for cutaneous squamous cell carcinoma (SCC) in immunocompetent individuals.

Design: Hospital-based case-control study.

Setting: Referral center for dermatologic diseases for central and southern Italy.

Participants: Consecutive patients with histologically confirmed cutaneous SCC (n=46) and control subjects (n=84) chosen by frequency matching (age and sex) among patients admitted with unrelated diseases.

Main Outcome Measures: Infection with epidermodysplasia verruciformis–related HPV types, blindly assessed by serologic testing (viruslike enzyme-linked immunosorbent assay). Information was obtained on known potentially confounding risk factors (family history, history and signs of sun exposure, and pigmenary traits) and on history of HPV-related lesions and diseases, assessed by interview and examination by a dermatologist.

Results: Positive serologic findings for HPV type 8 were associated with SCC (odds ratio, 3.2; 95% confidence interval, 1.3–7.9) independently of other risk factors, whereas positive serologic findings for HPV type 15 were negatively associated with SCC (odds ratio, 0.4; 95% confidence interval, 0.2–0.9). Other variables significantly associated with the tumor were family history of skin cancer, professional or recreational sun exposure, light eye color, high number of solar keratoses and seborrheic keratoses on the body surface, and residency in radon-emitting buildings.

Conclusions: Positive serologic findings for HPV type 8 are associated with SCC occurrence in immunocompetent individuals. Viral infection could act as a cofactor in the tumor development, along with genetic predisposition, solar radiation, and other environmental exposures. If confirmed, these findings could open new perspectives for treatment and prevention of SCC.

Arch Dermatol. 2003;139:890-894

HUMAN PAPILLOMAVIRUSES (HPVs) are small DNA viruses that infect epithelial cells and induce a variety of proliferative lesions, such as warts, laryngeal papillomas, and cervical carcinoma.1,2 In addition to their role in anogenital cancer,3-5 and probably in squamous cell carcinoma (SCC) of the head and neck,6-8 they are also associated with the development of skin cancer in individuals with epidermodysplasia verruciformis (EV), a rare inherited condition characterized by widespread HPV infection and eventual development of multiple SCCs, predominantly on sun-exposed sites.2

Epidermodysplasia verruciformis–associated and phylogenetically related HPV types (EV-HPV)7 are also frequently found in skin tumors of patients without EV. Immunosuppressed individuals, such as organ transplant recipients, seem to be especially prone to HPV infection. They develop numerous skin cancers, primarily SCC on sun-exposed sites, in the years after transplantation.8,10 It has also been shown that allograft recipients with high susceptibility to cutaneous malignancy have an increased prevalence of HPV DNA in skin tumors and a greater risk of anogenital malignancy, probably because of an increased susceptibility to persistent HPV infection due to iatrogenic immunosuppression.11

Despite the mounting body of epidemiologic evidence on the possible role of HPV in cutaneous oncogenesis, studies designed to assess the role of infection with EV-HPV in the development of cutaneous SCC are still rare and conducted mostly on rather small numbers of patients.12-20 Furthermore, as compared with anogenital cancers, there is little informa
tion so far on the molecular mechanisms involved in the HPV-dependent skin carcinogenesis.17

As antibodies to HPV capsid antigens are reliable markers of past or present HPV infection,18 and seroepidemiologic methods have been successfully used in studies that linked HPV infections to both anogenital and cutaneous lesions,19-23 we designed this seroepidemiologic study with the aim to evaluate EV-HPV infection as a risk factor for the development of cutaneous SCC in immunocompetent individuals.

SUBJECTS AND STUDY DESIGN

This case-control study was conducted in Rome at the Istituto Dermopatico dell’Immacolata, a referral dermatological center for central and southern Italy. The institutional ethical committee approved the study protocol before the study began.

Cases were subjects newly diagnosed as having histologically confirmed cutaneous invasive SCC in one surgical ward from October 1, 1999, to July 31, 2000, who gave their written informed consent to participate in the study. Control subjects were selected among patients with unrelated dermatologic conditions (mostly dermatitis, leg ulcers, alopecia, bullous diseases, and bacterial infections) admitted to 2 medical wards and were frequency matched to the cases by age and sex. Exclusion criteria for cases and controls were the presence of HPV-related skin lesions (warts and condylomas); history of SCC of the mucosa (cervix and head and neck); history or signs and symptoms of immunosuppression (including iatrogenic); and history of skin diseases for which UV radiation therapy was indicated, such as psoriasis or vitiligo, or sun avoidance was recommended, such as connective tissue diseases. Personal history of nonmelanoma skin cancer was an additional exclusion criterion for the controls.

A serum sample was obtained from each study subject, divided into aliquots, and stored at −20°C at Istituto Dermopatico dell’Immacolata until shipped in dry ice by air courier to Cologne, Germany, for testing.

With the use of a standardized questionnaire, detailed information was collected on demographic factors and housing: lifestyle (smoking, sexual habits, and attendance sport facilities such as swimming pools); history of professional and recreational sun exposure; history of sunburns; history of HPV-related skin lesions (warts, condylomas); and personal and family history of any cancer.

A complete skin examination was performed by a dermatologist to assess pigmentary characteristics (ie, eye color), the presence of sunlight-induced skin lesions (ie, solar keratoses, solar lentigines, and solar elastosis), and the number of seborrhic keratoses.

LABORATORY METHODS

The serologic response to the major capsid protein L1 of EV-HPV type 8 (EV-HPV-8) -15, -36, and -23 was tested by enzyme-linked immunosorbent assay with the use of viruslike particles (VLPs). Generation of HPV-8, HPV-15, and HPV-36 specific VLPs had been described previously.22,23 In case of HPV-23, the corresponding open reading frame was polymerase chain reaction–amplified by means of cloned reference template23 and primers 5’-TCCGTAGCTGCGACCCATAGCCCTCTGCCTCCAGCTTC-3’ (forward) and 5’-GGTCCGAGAAGCTTTTATGGCGACCGATATCGGTTAC-3’ (backward), which build in recognition sites for XhoI and HindIII, respectively. In addition, the translation-initiation signal in the forward primer was artificially optimized according to the data of Kozak.26 The cloning strategy, production, and purification of the VLPs were identical to that described by Wieland and coworkers.27

For each serum sample, the optical density (OD) value of an antigen-free well was subtracted from the mean value of duplicate wells coated with VLPs. A positive serum sample with an OD of 1.0 was included on each plate.

Individual serum samples were scored as antibody positive or negative by using a cutoff OD value based on the distribution of OD values of 291 healthy blood donors, aged 18 to 65 years. After individual values exceeding the mean +3 SD were excluded, the remaining readings were averaged again and the cutoff point was chosen at the 90th percentile of the OD value distribution observed in the healthy donors, to maximize specificity.

STATISTICAL ANALYSIS

A power calculation was performed before the study began, and was based on the hypothesis that 20% of the controls and 50% of the cases would have been seropositive for HPV-8. Considering a power of 0.80 and α = .05, a sample composed of 35 cases and 70 controls should have been enrolled.

All statistical analyses were performed with the statistical packages SPSS for Windows, release 8.0.28 and Stata for Windows, release 4.0.29 Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for known risk factors (family history of skin cancer, light eye color, sunlight-related skin lesions), occupational and recreational sun exposure, history of sunburns, lifestyle-related habits (smoking and housing conditions), serologic findings for EV-HPV, and history of HPV-related diseases.

Unconditional logistic regression models were built to allow for multiple adjusting of confounding and thus to identify variables independently associated with SCC.

In all models, age was considered as a continuous variable. For categorical variables, point estimates were first computed for each category. Adjacent categories for which the calculated ORs were very similar were collapsed in a single category whenever possible, to increase robustness of the estimates. Variables that were measured on a continuous scale were categorized by dichotomizing or dividing in tertiles the distributions of exposed controls, and, where appropriate, adjacent categories were eventually collapsed, as described.

RESULTS

At the end of the study, questionnaires, clinical information, and corresponding serum samples were available for 46 cases and 84 controls. Of 51 clinically eligible case subjects, 2 refused to participate in the study, and in 3 cases the clinical diagnosis was not confirmed by histologic examination. For the enrolled cases, the location of SCC was the lip (n=13), other regions of the head (n=27), or other body areas (n=6). Of 137 eligible controls, 52 refused to participate in the study and 1 was erroneously not requested to donate serum samples.

The demographic characteristics of the study subjects are summarized in Table 1. As expected, cases were mainly elderly men (mean age, 72 years), and no differences in age and sex were observed between cases and controls, indicating that frequency matching on these 2 variables was successful. No differences were observed between cases and controls for place of residency.

The known risk factors for SCC (family history of skin cancer, sun exposure, and light eye color) and sun-
induced skin lesions, such as solar keratoses, were strongly associated with disease in our study population (Table 2). The risk of developing SCC was increased also for people with 10 or more seborrheic keratoses on body surfaces and for people who lived in radon-emitting tuff buildings.

Of the 4 EV-HPVs for which antibodies were sought in the serum samples of the study subjects (Table 3), HPV-8 and HPV-36 (members of subgroup A of EV-HPV) were associated with SCC, while HPV-15 and HPV-23 (subgroup B) were not, although the small number of patients positive for HPV-36 and HPV-23 did not allow any inference (Figure). Positive serologic findings for HPV-8 were also associated with male sex (OR, 3.3; 95% CI, 1.5-7.5).

In multivariate analysis, positive serologic findings for HPV-8 were confirmed to be associated with SCC, independently of age and sex, other known risk factors (sun exposure [as assessed by profession, history of exposure, and sunburns] and pigmentary traits [as assessed by eye color]), and other variables of interest such as number of seborrheic warts and radon exposure.

Positive serologic findings for HPV-15 were shown to be negatively associated with SCC, and this was confirmed in multivariate analysis.

**COMMENT**

The main finding of our study in immunocompetent individuals was that subjects with positive serologic findings for HPV-8 had a 3-fold risk of cutaneous SCC (OR, 3.2; 95% CI, 1.3-7.9), independently of other risk factors. This association is in accordance with previous findings of HPV-specific DNA sequences in tumoral tissue and in eyebrow hairs of immunocompetent individuals with SCC. Two previous seroepidemiologic studies performed with the same method used in this study showed a high prevalence of antibodies against HPV-8 in patients with SCC (10 positive results in 14 patients compared with about 7% in the general German population) and an association of seropositivity for HPV-8 with the development of actinic keratoses.
(OR, 2.4; 95% CI, 1.1-5.0) and invasive SCC (OR, 2.7; 95% CI, 0.7-11.6) in the population of the tropical island of Saba.23

Our study confirms and strengthens these previous results, as it is based on an adequate sample size and the study participants were recruited in a Mediterranean country (Italy), where genetic and phenotypic characteristics and patterns of solar exposure are quite different from those of previously studied populations.

A possible bias could have been introduced by the high number of potential controls unwilling to participate in the study (52 of 137). However, we carefully matched the cases and controls by age and sex. The controls unwilling to participate were very similar in sex, residence, and skin diseases to those who accepted, but they were significantly older and probably had a lower educational level; this, however, was not associated with HPV-8 infection in the participating controls.

As we expected a high mean age in the study population, pigmented traits were assessed only by recording eye color, and solar exposure was investigated by simple questions on the lifetime amount of professional or recreational sun exposure (high or low) and history of sunburns, to avoid asking more detailed questions that would have resulted in unreliable information.20-31

By logistic regression, positive serologic findings for HPV-8 were independently associated with SCC occurrence in all models tested, with only slight differences in the point estimates. The adjusted ORs shown in Table 3 are derived from the most parsimonious model, which included only the exposure of interest and the main known risk factors, to minimize inflation of the point estimates and CIs.

Also, for HPV-36, a virus type that belongs to the same subgroup of EV-HPV as HPV-8,9 we observed an adjusted OR of 2.8, suggesting a possible association with SCC. However, because of the low prevalence of HPV-36 in our population, the power of our study (which was calculated on the basis of the expected prevalence of HPV-8) was insufficient to reach statistical significance.

By contrast, seropositivity for HPV-15 was negatively associated with SCC. Our finding that an excess risk was associated only with HPV-8 and possibly with HPV-36, but not with the other EV-HPV types we tested (even if they probably share similar modes of transmission), suggests that the HPV-associated risk is not confounded by differences in lifestyle. Instead, it seems plausible that for cutaneous SCC, like what is known about cervical SCC, high-risk oncogenic HPV types may exist, and also that infection with low-risk HPV types may confer protection against the development of the tumor, probably through immunomediared mechanisms.32

The advantages and limitations of determining HPV infection by detecting antibody response to HPV capsids have been extensively discussed in previous articles by our group32 and others.33 As the VLP–enzyme-linked immunosorbent assay is able to detect present as well as past infections, its validity does not depend on a precise sampling of the tissue, and no obvious cross-reactivity between different HPV types is known, we believed that this method could be particularly useful for the preliminary assessment of cancer risk. Because the viral genome is maintained and expressed in the lesions in most cervical SCC and EV skin cancers, further studies assessing the presence of viral DNA and RNA also in cutaneous SCC in immunocompetent individuals would be needed to prove that HPV-8 may be causally involved in skin malignancy.13 However, we do not know at present whether expression of the viral genome needs to be maintained or is necessary only in the early steps of malignant transformation in cutaneous carcinogenesis.

Moreover, epidermal proliferation limited to small cutaneous areas, such as nonmelanoma skin cancer, was not found sufficient to evoke anti–HPV-5 antibody formation as a marker of process linked to epidermal proliferation, as in conditions implying extensive reepithelization.34 Similarly, it seems difficult to consider HPV-8 antibody formation simply as a marker of process in cutaneous carcinogenesis, especially in immunocompetent individuals.

The presence of 10 or more solar keratoses was strongly associated with the presence of the tumor (OR, 42.8; 95% CI, 5.4-339.2), and all 8 HPV-8–positive subjects with 10 or more solar keratoses had SCC. This result was not surprising, confirming that solar keratoses are to be considered an SCC “in situ,” undergoing a long precancerous phase before progressing to invasive carcinoma. However, association between HPV-8 positive serologic findings and presence of 10 or more solar keratoses was not significant (OR, 1.63; 95% CI, 0.57-4.69).

The strong association of seborrheic keratoses with SCC was an unexpected finding. We recorded this variable to verify a reported association with HPV infection35 and sun exposure.20 In fact, the presence and number of

---

Distribution of L1 antibodies for human papillomavirus type 8 (HPV-8), HPV-15, and HPV-23 in 46 patients with squamous cell carcinoma, 84 age- and sex-matched control subjects, and 291 healthy blood donors. OD indicates optical density in the enzyme-linked immunosorbent assay reader at 405 nm. P values by analysis of variance between cases and controls were .01 for HPV-8, .09 for HPV-15, and .44 for HPV-23.
seborrheic keratoses was not associated with seropositivity for any of the HPV types tested, while it was associated with increasing age, history of sunburns and number of solar keratoses, family history of skin cancer, and presence of SCC. On the basis of these findings, a high number of seborrheic warts (>10) on the body surface might probably be regarded as a risk marker for SCC occurrence.

Finally, another interesting and unexpected finding was the association between SCC and living in tuff buildings, independently of other risk factors for the tumor and of other socioeconomic indicators (OR, 3.1; 95% CI, 1.2—7.9—adjusted for age, sex, sun exposure, eye color, and HPV-8 serologic findings). Tuff is a building material widely used in central Italy; it originates from volcanic rocks and has a high potential for radon emission.27—30 Although this might be a chance finding, since important cohort studies have not shown excess mortality from skin cancers in underground miners exposed to high radon concentrations, it is possible that environmental radon exposure may interplay with other factors to which miners may be underexposed (eg, solar radiation) in increasing the risk of SCC.29—32

Our study had insufficient power to estimate the possible interactions between different risk factors, and further studies are needed to determine whether a number of “high-risk” cutaneous HPV strains are associated with SCC and how these agents might intervene in the induction of the tumor, most probably acting as a cofactor along with genetic predisposition, solar radiation, and other environmental exposures.

Accepted for publication November 27, 2002. This study was supported in part by the “Ricerca Corrente” Program of the Italian Ministry of Health, Rome, 2000. Drs Fuchs and Pfister were supported by a grant from the Center for Molecular Medicine, Cologne University, Cologne. This study was presented in part as a poster at the World Congress of Dermatology, Paris, France, July 1-3, 2002. Corresponding author and reprint requests: Cinzia Masini, MD, Clinical Epidemiology Unit, Istituto Dermopatico dell’Immacolata (IDI-IRCCS), Via Monti di Creta 104, 00167 Rome, Italy (e-mail: c.masini@idi.it).

REFERENCES