HUMAN PAPILLOMAVIRUS IN BASAL CELL CARCINOMA – A PILOT STUDY

THOMAS IFTNER,[1] ANGELIKA IFTNER,[1] PETRA G BUETTNER,[2] SIMONE L HARRISON,[2] MARGARET GLASBY,[2] BEVERLY A RAASCH[3] and CLAUS GARBE[4]

[1]Research Section Experimental Virology, Institute of Medical Virology, Eberhard-Karls University, Tübingen, Germany; [2]Skin Cancer Research Group within the North Queensland Centre for Cancer Research, School of Public Health, Tropical Medicine and Rehabilitation Sciences, James Cook University, Townsville, Australia; [3]Department of Family Medicine, Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates; and [4]Department of Dermatology, Eberhard-Karls University, Tübingen, Germany.

Corresponding author: Petra.Buttner@jcu.edu.au

ABSTRACT

Objectives: The study investigated whether test results for human papillomavirus (HPV) are influenced by the sampling site of the specimens. Methods: The present study was a pilot study for a large case-control study currently been conducted and included 14 immune-competent north Australian patients with histologically confirmed basal cell carcinoma. Specimens assessed were the cancer tissue, peri-lesional skin, healthy skin, and eyebrow hairs. HPV types were identified by single-tube nested 'hanging droplet' polymerase chain reaction. Results: HPV was isolated from four patients (28.6%; 95%-confidence interval = [4.0, 32.7]) with up to three of the samples from these patients testing positive for HPV. The prevalence of HPV and the types involved varied with the sampling site. All healthy skin samples were negative for HPV. Conclusions: HPV results varied considerably with the sample site in patients with basal cell carcinoma. Therefore, the authors suggest that standardising specimen collection for HPV analysis to the cancer site should reduce the possibility of detecting HPV types unrelated to the development of skin cancer.

KEY WORDS: Human papillomavirus; Basal cell carcinoma; Skin cancer; Standardisation of specimen collection; Aetiology.

ACCEPTED: 16 September 2009

INTRODUCTION

Human papillomaviruses (HPVs) are increasingly recognized as important human carcinogens, particularly with respect to anogenital (cervical) cancer (Bosch et al., 2002; zur Hausen 1996). HPV has been also implicated in the aetiology of non-melanoma skin cancer, in particular for squamous cell carcinoma, while the role of HPV in the development of basal cell carcinoma (BCC) seems less clear (Iftner et al., 2003; Karagas et al., 2006).

Various sampling techniques have been used to study the relationship between HPV and skin cancer: for example, Karagas and colleagues (2006) used plasma samples, Iftner and colleagues (2003) used biopsies from the cancer site, while other investigators additionally or solely analysed peri-lesional skin (Forslund et al., 2003a), eyebrow hairs (Struijk et al., 2003) and normal skin biopsies (Forslund et al., 2003a; Harwood et al., 2004). The present study investigated whether the sampling site could influence the results of the HPV analysis.

METHODS

During the pilot of a large case-control study examining the aetiology of basal cell carcinoma, snap frozen (-70oC) skin biopsies of cancer tissue from 14 immune-competent patients with histologically confirmed BCC were analysed for HPV. Other samples collected from these patients for HPV analysis included peri-lesional skin (available for 7 subjects), healthy skin (9 subjects), and eyebrow hairs (11 subjects). Participants were all residents of a regional town in tropical Australia (latitude 19S). Ethical approval was gained from James Cook University Human Ethics Committee and from the Townsville Health District Ethics Committee.

The single-tube nested 'hanging droplet' polymerase chain reaction (PCR) was performed as described by Forslund and colleagues (2003b). As described previously, a broad spectrum of human papillomavirus types is present in the skin of Australian patients with non-melanoma skin cancers and solar keratosis (Forslund et al., 2003a). The nested FAP primer PCR system has been shown to amplify a broad spectrum of HPV types from the skin of both humans and animals. The detection limit has been determined to approximately 10 copies of HPV 5 and HPV 30, and one copy of HPV 20. However, amplification is unsuccessful for cutaneous HPV types 1, 2, 41 and 63. All samples were tested for integrity of the DNA by PCR using primers for the human ß-globin gene. All PCR amplicons were cloned and sequenced: at least three clones from each sample were usually obtained using the TOPO TA CloningKit (Invitrogen, Leek, The Netherlands). The nucleotide sequences were generated via cycle sequencing (ABI Prism BigDye Terminator Cycle Sequencing, Applied Biosystems, California) and an automated DNA sequencer (310 Genetic Analyzer, Applied Biosystems). The sequences were compared with the GenBank database by the Blast program (http://www.ncbi.nlm.nih.gov/BLAST/). A nucleotide sequence was regarded as a distinct HPV type if it shared

90% or more homology with a known type. Sequence homologies below 90% were regarded as related types.

HPV taxonomy was based on the criteria established by the International Committee on the Taxonomy of Viruses (de Villiers et al., 2004). Results of HPV analyses were related to epidemiological data collected using self-administered questionnaires. Pack-years of cigarette smoking were calculated as the number of packs of cigarettes smoked per day multiplied by the number of years of smoking.

RESULTS

All participants (eleven males; median age 60 years; age range 37 - 76 years) were born in Australasia and were of northern European descent. All participants judged their own skin colour to be fair, and 81.8% said that their skin would always burn if unprotected for 30 minutes in strong sunlight for the first time in summer (three missing values). All participants had had previous skin cancer excisions.

Of the 14 participants, four (28.6%; 95%-confidence interval = 95%-CI = [4.0, 32.7]) provided at least one sample that tested positive for HPV (Table). Two of 14 (14.3%; 95%-CI = [1.8, 42.8]) BCC biopsies were positive for HPV [genus beta: species 2 (HPV9 & HPV80); genus alpha: species 7 (HPV45) and species 9 (HPV52)]. Two of the seven peri-lesional biopsies (28.6%; 95%-CI = [3.7, 71.0]) and two of the 11 eyebrow hair samples (18.2%; 95%-CI = [2.3, 51.8]) were HPV positive. Only one subject (61-year old male) was HPV positive for the BCC biopsy, the peri-lesional biopsy, and the eyebrow hair sample. All HPV detected within these three samples were of the genus beta, and while most were species 2, species 1 was also present, with varying HPV types. None of the nine healthy skin samples (95%-CI = [0, 33.6]) tested positive for HPV.

All four participants with HPV positive samples were male. None of the two non-smokers, two of the three ex-smokers and both current smokers had HPV positive samples (n=7, 7 missing as not all patients were sampled for all sites, exact p = 0.314). The median number of pack-years of smoking cigarettes was 0 (inter-quartile range = 0, 4) for HPV negative and 39 (inter-quartile range = 18.8, 53.3) for HPV positive patients (p = 0.032).

Table: Results of identification of genus and species of human papillomaviruses (HPV) in 14 immune-competent Australian patients with basal cell carcinoma.

Patient	Cancer tissue	Peri-lesional skin	Eyebrow hair	Healthy skin
1	None	None	/	/
2	None	/	/	/
3	None	None	/	/
4	Genus beta: species 2 (HPV9 & HPV80)	Genus beta: species 1 (HPV12) and species 2 (HPV15)	Genus beta: species 2 (HPV9)	None
5	None	/	Genus beta: species 1 (HPV14) and species 2 (HPV17 & HPV22)	None
6	None	None	None	None
7	None	/	None	/
8	None	None	None	None
9	None	/	None	None
10	Genus alpha: species 7 (HPV45) and species 9 (HPV52)	/	None	/
11	None	/	None	None
12	None	/	None	None
13	None	None	None	None
14	None	Genus gamma: species 1 (HPV65) and species 3 (HPV48)	None	None

DISCUSSION

The prevalence of HPV in this limited sized pilot study of patients with basal cell carcinoma was relatively low, ranging between 0% and almost 30% depending on the site sampled. In comparison, the prevalence of infection with HPV in patients with squamous cell carcinoma was found to be higher than 80% in some studies (Berkhout et al., 2000; Orth et al., 1980).

Within positive patients, HPV species varied or HPV was found at one site but not at other sites. Different HPV types of the genera alpha, beta and gamma were detected for each positive case; a diversity which has been reported previously (Forslund et al., 2003a). There was no dominance of epidermodysplasia verruciformis-HPV types (genus beta). It has been previously described that patients with the rare genetic disorder epidermodysplasia verruciformis develop flat HPV-induced warts, which progress to become primary SCCs in up to 60% of cases (Pfister 1992). The present results call for a uniform approach to specimen collection for HPV analysis, as

findings varied with the sampling site. The authors suggest standardising specimen collection for HPV analysis to the cancer tissue, which would eliminate linking potentially unrelated HPV infections with the development of skin cancer.

Despite the very small sample size, the present data suggest that in BCC, infection with HPV could be related to smoking. While the role of smoking in the development of BCC remains inconclusive (Freedman et al., 2003; Milan et al., 2003), one possible explanation for the finding is that infection with HPV in BCC is merely the result of immune suppression caused inter alia by smoking, in a manner unrelated to the cancer. There is a need for future molecular-epidemiological studies to further clarify these relationships.

REFERENCES

Berkhout RJ, Bouwes Bavinck JN, ter Schegget J. (2000) Persistence of human papillomavirus DNA in benign and (pre)malignant skin lesions from renal transplant recipients. *Journal of Clinical Microbiology* 38: 2087-96.

Bosch F, Lorincz A, Munoz N, Meijer C, Shah K. (2002) The causal relation between human papillomavirus and cervical cancer. *Journal of Clinical Pathology* 55: 244-65.

de Villiers E, Fauquet C, Broker T, Bernard H, zur Hausen H. (2004) Classification of papillomaviruses. *Virology* 324(1):17-27.

Forslund O, Ly H, Reid C, Higgins G. (2003a) A broad spectrum of human papillomavirus types is present in the skin of Australian patients with non-melanoma skin cancers and solar keratosis. *British Journal of Dermatology* 149(1):64-73.

Forslund O, Ly H, Higgins G. (2003b) Improved detection of cutaneous human papillomavirus DNA by single tube nested 'hanging droplet' PCR. *Journal of Virology Methods* 110(2): 129-36.

Freedman D, Sigurdson A, Doody M, Mabuchi K, Linet M. (2003) Risk of basal cell carcinoma in relation to alcohol intake and smoking. *Cancer Epidemiology Biomarkers and Prevention* 12(12):1540-3.

Harwood C, Surentheran T, Sasieni P, Proby C, Bordea C, Leigh I, Wojnarowska F, Breuer J, McGregor J. (2004) Increased risk of skin cancer associated with the presence of epidermodysplasia verruciformis human papillomavirus types in normal skin. *British Journal of Dermatology* 150(5): 949-57.

Iftner A, Klug S, Garbe C, Blum A, Stancu A, Wilczynski S, Iftner T. (2003) The prevalence of human papillomavirus genotypes in nonmelanoma skin cancers of nonimmunosuppressed individuals identifies high-risk genital types as possible risk factors. *Cancer Research* 63(21): 7515-9.

Karagas M, Nelson H, Sehr P, Waterboer T, Stukel T, Andrew A, Green A, Bavinck J, Perry A, Spencer S, Rees J, Mott L, Pawlita M. (2006) Human papilloma-virus infection and incidence of squamous cell and basal cell carcinomas of the skin. *Journal of the National Cancer Institute* 98(6): 389-95.

Milan T, Verkasalo P, Kaprio J, Koskenvuo M. (2003) Lifestyle differences in twin pairs discordant for basal cell carcinoma of the skin. *British Journal of Dermatology* 149(1):115-23.

Orth G, Favre M, Breitburd F. (1980) Epidermodysplasia verruciformis: a model for the role of papillomaviruses in human cancer, pp. 259-282. In: Viruses in naturally occurring cancers, Vol 7, (eds.) Essex M, Todaro G, zur Hausen H. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory, New York, United States of America.

Pfister H. Human papillomaviruses & skin cancer. Seminars in Cancer Biology 1992; 3: 263-71.

Struijk L, Bouwes Bavinck J, Wanningen P, van der Meijden E, Westendorp R, Ter Schegget J, Feltkamp M. (2003) Presence of human papillomavirus DNA in plucked eyebrow hairs is associated with a history of cutaneous squamous cell carcinoma. *Journal of Investigative Dermatology* 121(6): 1531-5.

Zur Hausen H. (1996) Papillomavirus infection - a major cause of human cancers. *Biochemical Biophysical Acta* 1288(2): F55-F78.

