

Re: Human Papillomavirus DNA and p53 Polymorphisms in Squamous Cell Carcinomas From Fanconi Anemia Patients

Kutler et al. (1) recently reported that human papillomavirus (HPV) DNA is frequently detected in squamous cell carcinomas (SCCs) of Fanconi anemia patients, who are predisposed to cancer at an early age, particularly acute myeloid leukemia and SCCs of the head and neck (HNSCCs) and the oral cavity region. Kutler et al. speculate that HPV infection might be critically involved in the pathogenesis of SCC in Fanconi anemia patients, which raises hopes that vaccination against HPV might prevent such tumors. HPV is known to be etiologically involved in SCCs that arise in the anogenital region (cervix) and in a minority of HNSCCs, particularly those in the oropharynx (2,3). Inhibitory oncoproteins expressed by HPV cause inactivation of the p53 and pRb pathways. HNSCCs that contain transcriptionally active HPV DNA typically lack mutations in TP53, the gene encoding p53, whereas 50%–60% of HNSCCs that do not contain HPV DNA have mutations in TP53 (2).

Kutler et al. (1) report that 15 of the 18 HNSCCs (all in the oral cavity) from the Fanconi anemia patients in their study had detectable HPV DNA and that none of the 18 HNSCCs contained a mutation in TP53. HPV DNA was detected by polymerase chain reaction (PCR) in laser-microdissected tissue, and quantitative PCR was used to determine the HPV DNA copy number per tumor cell. A cutoff value set at more than one copy per 10 cells was used to designate a sample positive for HPV DNA.

We have collected similar information on HNSCCs from Fanconi anemia patients and have obtained different results. Of the five HNSCCs studied, none were HPV DNA-positive and four had a

mutation in TP53. Although we studied fewer tumors than Kutler et al., the frequencies of HPV DNA positivity and TP53 mutations are statistically significantly different when comparing their results with ours ($P = .002$ for presence of HPV DNA, and $P < .001$ for mutations in TP53). The basis for these differences is unclear. They may reflect differences in ethnic backgrounds or life-style factors (i.e., tobacco and/or alcohol use, disease history, or sexual behavior) between the patients we studied and those studied by Kutler et al. In addition to patient-related differences, slight differences in HPV DNA detection methodologies might account for part of the discrepancy between our results and those of Kutler et al. We used a semiquantitative PCR-based method with consensus primers and an oligoprobe cocktail to detect the DNA of 14 high-risk HPV types in total tumor DNA; this method can detect as few as one copy of HPV16 DNA per 5000 cells (4). However, none of the HNSCC specimens from the Fanconi anemia patients we tested were positive for HPV DNA, which made further quantitative analysis redundant. Results of previous studies suggest that the likely involvement of HPV in the etiology of a tumor is reflected by the presence of at least one copy of HPV DNA in every cancer cell (2,5). To obtain independent confirmation of the HPV DNA status of HNSCCs from Fanconi anemia patients, we suggest that loss-of-heterozygosity patterns in the tumor DNA could be evaluated, because we have found that HNSCCs with transcriptionally active HPV DNA appear to exhibit a loss-of-heterozygosity pattern that is strikingly different from that observed among HNSCCs without detectable HPV DNA (6).

Until these issues have been examined in more detail, we wish to caution against raising too much hope that HPV vaccination (7) will be an option to prevent HNSCC in Fanconi anemia patients. The current policy of frequent surveillance and examination of biopsy samples of suspected tissues in the oral cavity and oropharynx should remain part of the standard practice in the clinical management of Fanconi anemia patients, including those who are enrolled in preventive vaccination trials.

HESTER J. T. VAN ZEEBURG
PETER J. F. SNIJDERS

HANS JOENJE

RUUD H. BRAKENHOFF

REFERENCES

- (1) Kutler DI, Wreesmann VB, Goberdhan A, Ben-Porat L, Satagopan J, Ngai I, et al. Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *J Natl Cancer Inst* 2003;95:1718–21.
- (2) van Houten VM, Snijders PJ, van den Brekel MW, Kummer JA, Meijer CJ, van Leeuwen B, et al. Biological evidence that human papillomaviruses are etiologically involved in a subgroup of head and neck squamous cell carcinomas. *Int J Cancer* 2001;93:232–5.
- (3) Gillison ML, Koch WM, Capone RB, Spaford M, Westra WH, Wu L, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst* 2000;92:709–20.
- (4) van den Brule AJ, Pol R, Franssen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J Clin Microbiol* 2002;40:779–87.
- (5) Ha PK, Pai SI, Westra WH, Gillison ML, Tong BC, Sidransky D, et al. Real-time quantitative PCR demonstrates low prevalence of human papillomavirus type 16 in premalignant and malignant lesions of the oral cavity. *Clin Cancer Res* 2002;8:1203–9.
- (6) Braakhuis BJ, Snijders PJ, Keune WH, Meijer CJ, Ruijter-Schippers HJ, Leemans CR, et al. Genetic patterns in head and neck cancers that contain or lack transcriptionally active human papillomavirus. *J Natl Cancer Inst*. In press 2004.
- (7) Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002;347:1645–51.

NOTES

Affiliations of authors: Department of Otolaryngology/Head-Neck Surgery (HJTVZ, RHB), Department of Pathology (PJFS), and Department of Clinical Genetics and Human Genetics (HJ), Vrije Universiteit Medical Center, Amsterdam, the Netherlands.

Correspondence to: R. H. Brakenhoff, PhD, Tumor Biology Section, Department of Otolaryngology/Head-Neck Surgery, Vrije Universiteit Medical Center, P.O. Box 7057, 1007 MB, Amsterdam, The Netherlands (e-mail: rh.brakenhoff@vumc.nl).

DOI: 10.1093/jnci/djh178

RESPONSE

We reported a high prevalence of human papillomavirus (HPV) DNA and the absence of p53 mutations among 24 squamous cell carcinomas (SCCs) from 25 Fanconi anemia patients (1). By con-

trast, van Zeeburg et al. describe an unpublished series of five cases of head and neck squamous cell carcinoma (HNSCC) from Fanconi anemia patients in which they identified mutations in p53 in four cases and the absence of detectable HPV DNA in all cases.

Several factors may explain the different results obtained in these two studies, the least persuasive of which are the small sample size in the van Zeeburg et al. study and methodologic differences between the studies, including the source of DNA (we used laser capture microdissected specimens), the oligonucleotide primers used for HPV DNA detection (we used highly efficient CPI and CPIIG consensus primers and validated all findings with real-time polymerase chain reaction), and stringency of analyses performed (our experiments were repeated at least three times) (2,3).

One of the more important reasons for the differing results may be case selection. We included all cases of SCC reported in the International Fanconi Anemia Registry (IFAR) without restrictions. Conversely, the cases van Zeeburg et al. report on were presumably selected on the basis of the authors' ability to establish long-term tumor cell cultures (at least for four of the five reported cases). This is an important distinction, because SCCs from Fanconi anemia patients are known to be difficult to put into culture, and therefore the cases reported by van Zeeburg et al. may not be representative of the HNSCCs found in Fanconi anemia patients.

A major clinical difference between SCCs in Fanconi anemia patients and those in the general population is the lower prevalence of tobacco and/or alcohol use among Fanconi anemia patients, which is known to have a substantial impact on the pattern of genomic aberrations in SCCs (4). Although van Zeeburg et al. agree that this is an important issue, they do not provide the carcinogen exposure histories for their case patients. Interestingly, one of the authors has previously published data on the absence of detectable HPV DNA in two of the five cases included in the current series (5). In that study, both cases of HNSCC occurred in patients who had a clinically significant history of tobacco or alcohol exposure, which sharply contrasts with the tobacco and/or alcohol exposures of most Fanconi anemia patients who develop SCC (5).

Our unpublished observations suggest that the incidence, pathogenesis, and genomic composition of Fanconi anemia-associated HNSCC that develop in patients who suffer graft-versus-host disease after having a bone marrow transplant may be different from those occurring in the absence of this disease (Kutler DI, Wreesmann VB, Goberdhan A, Ben-Porat L, Satagopan J, Ngai I, et al.: unpublished observations). In this respect, it is interesting to note that the rate of graft-versus-host disease is historically higher in Europe than in the United States due to differences in transplantation protocols. This clinical information should also be provided by van Zeeburg et al. to allow a meaningful assessment of their results.

Finally, on the basis of their unpublished findings, van Zeeburg et al. suggest that a unique pattern of loss-of-heterozygosity may help identify HPV DNA-positive HNSCCs. However, there are several problems with this approach. Fewer than 10% of patients with HNSCC in the general population report having no history of tobacco or alcohol use. Accordingly, the substantial amount of tobacco and/or alcohol consumption has complicated delineation of the contributions of HPV to the pathogenesis of HNSCC in the general population, not to mention the identification of reproducible differences in the genetic composition in HPV-related and HPV-unrelated cases of HNSCC. We performed a genomic screening study comparing all cases of HNSCC in Fanconi anemia patients (N = 18) with a group of randomly selected cases of HNSCC from the general population (n = 42; Kutler DI, Wreesmann VB, Goberdhan A, Ben-Porat L, Satagopan J, Ngai I, et al.: unpublished data). We found statistically significant differences in the pattern and frequency of chromosomal aberrations detected by comparative genomic hybridization between HNSCCs from the two populations. This result differs considerably from that in a previous report on two of the five cases in the authors' series, in which no differences in chromosomal aberrations were found between the HNSCCs in two Fanconi anemia patients and those in the general population, reflecting the selection bias for the cases in their series (5).

Overall, the high prevalence of HNSCC in Fanconi anemia patients re-

quires diligent surveillance of this patient population. On the basis of our findings, the role of HPV in the pathogenesis of HNSCC in Fanconi anemia patients merits further investigation, as does HPV vaccination as a mode of cancer prevention.

DAVID I. KUTLER
VOLKERT B. WREESMANN
BHUVANESH SINGH

REFERENCES

- (1) Kutler DI, Wreesmann VB, Goberdhan A, Ben-Porat L, Satagopan J, Ngai I, et al. Human papillomavirus DBA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. *J Natl Cancer Inst* 2003;95:1718–21.
- (2) van den Brule AJ, Pol R, Franssen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J Clin Microbiol* 2002;40:779–87.
- (3) Ha PK, Pai SI, Westra WH, Gillison ML, Tong BC, Sidransky D, et al. Real-time quantitative PCR demonstrates low prevalence of human papillomavirus type 16 in premalignant and malignant lesions of the oral cavity. *Clin Cancer Res* 2002;8:1203–9.
- (4) Brennan JA, Boyle JO, Koch WM, Goodman SN, Hruban RH, Eby YJ, et al. Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. *N Engl J Med* 1995;332:712–7.
- (5) Hermsen MA, Xie Y, Rooimans MA, Meijer GA, Baak JP, Plukker JT, et al. Cytogenetic characteristics of oral squamous cell carcinomas in Fanconi anemia. *Fam Cancer* 2001;1:39–43.

NOTES

Affiliation of authors: Laboratory of Epithelial Cancer Biology, Memorial Sloan-Kettering Cancer Center, New York, NY.

Correspondence to: Bhuvanesh Singh, MD, Laboratory of Epithelial Cancer Biology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021 (e-mail: singhb@mskcc.org).

DOI: 10.1093/jnci/djh179