Letter to the Editor

Human Papillomavirus Integration Is Not Associated with Advanced Epithelial Ovarian Cancer in German Patients

Ingo B. Runnebaum, Sabine Maier, Xiao W. Tong, Heike E. Rosenthal, Volker J. Möbus, Dirk G. Kieback and Rolf Kreienberg


Received 12/15/94; revised 3/6/95; accepted 3/7/95.

Oncogenic forms of the HPV are frequently found in the lower female genital tract, presumably leading to the inactivation of the tumor suppressor p53 (1). In Germany, oncogenic human papillomavirus types 16 and 18 have been detected in the cytologically normal lower genital tract of at least 15% of the female population (2). HPV infection of ovarian epithelial cells could therefore be suspected to be involved in the development of some ovarian cancers because 75–90% of ovarian malignancies derive from the ovarian epithelium. Conflicting data have been reported on the presence of HPV sequences in ovarian cancer (3–7). We have screened 37 permanent cell lines established from advanced human ovarian cancer, as well as 28 paraffin-embedded tissues of advanced primary ovarian carcinomas, all from German Caucasians, for the presence of HPV sequences.

Ovarian cancer cell lines were established from serous adenocarcinoma of the ovary as published elsewhere (8). Genomic DNA of the cell lines was extracted following standard protocols. Genomic DNA from the cervical cancer cell lines HeLa, CaSki, and SiHa was generously provided by Dr. Matthias Dürst, [German Cancer Research Center (Deutsches Krebsforschungszentrum), Heidelberg, Germany]. The paraffin-embedded tissues from primary tumor sites containing more than 70% malignant cells were deparaffinized with the use of octane and subjected to proteinase K digestion as described elsewhere (9). Tissues included in this study were taken at the time of surgery from German Caucasians. DNA was amplified from HeLa (Fig. 2) and SiHa with various dilutions of HeLa and SiHa template.

Genomic DNA of the cell lines was extracted following standard protocols. Genomic DNA from the cervical cancer cell lines HeLa, CaSki, and SiHa was generously provided by Dr. Matthias Dürst, [German Cancer Research Center (Deutsches Krebsforschungszentrum), Heidelberg, Germany]. The paraffin-embedded tissues from primary tumor sites containing more than 70% malignant cells were deparaffinized with the use of octane and subjected to proteinase K digestion as described elsewhere (9). Tissues included in this study were taken at the time of surgery from German Caucasians. DNA was amplified from HeLa (Fig. 2) and SiHa with various dilutions of HeLa and SiHa template.

Differentiation carcinomas, 1 clear cell cystadenocarcinoma, and 1 granulosa cell tumor. The histopathological types of the tumors giving rise to the ovarian cancer cell lines of this study were: 27 serous cystadenocarcinomas, 1 endometrioid adenocarcinoma, 2 mucinous cystadenocarcinomas, 1 mixed endometrioid and serous cystadenocarcinoma, 1 heterologous malignant mixed Müllerian tumor, 1 undifferentiated carcinoma, and 1 granulosa cell tumor. The histopathological types of the tumors giving rise to the ovarian cancer cell lines of this study were: 27 serous cystadenocarcinomas, 1 endometrioid adenocarcinoma, 2 mucinous cystadenocarcinomas, 1 mixed endometrioid and serous cystadenocarcinoma, 1 heterologous malignant mixed Müllerian tumor, 1 undifferentiated carcinoma, and 1 granulosa cell tumor.

In 28 ovarian cancer tissues, no specific amplification product was detected by agarose gel electrophoresis with the use of L1 consensus primer PCR. Genomic DNA from the ovarian cancer cell lines with either wild-type (15 of 37) or mutant (22 of 37) p53 also did not permit specific amplification with L1 consensus primers. HeLa, CaSki, and SiHa DNA samples reliably lead to strong signals already in this assay (Fig. 1). To increase the assay sensitivity, a dot blot analysis was carried out. L1 PCR products were transferred to a nylon membrane and hybridized with a generic probe (Fig. 2). Specific hybridization was not observed for tissue specimens or ovarian cancer cell lines, including those carrying a wild-type p53 sequence. PCR products derived from HeLa (Fig. 2) and SiHa templates yielded strong signals. The sensitivity of the dot blot assay system was calculated to be approximately 1 HPV genome/host genome after setting up the L1 consensus PCR with various dilutions of HeLa and SiHa template.

E6 multiplex PCR amplification from HeLa cells lead to a 358-bp PCR product indicative of E6 of HPV 18, whereas SiHa

© 1995 American Association for Cancer Research. cebp.aacrjournals.org Downloaded from
cells allowed the amplification of a 323-bp fragment from the E6 of HPV 16. Neither the tumor tissues nor the ovarian cancer cell lines proved to be positive for the presence of HPV 16 or HPV 18 E6 oncogene (Fig. 3).

Molecular epidemiology has identified human papillomavirus infection of the lower female genital tract as a major contributing factor to intraepithelial neoplasia and cancer of the vulva and uterine cervix. The figures for the prevalence of HPV infection in healthy, sexually active women vary depending on the type of assay applied. In the United States a prevalence of up to 46% of subclinical infection of vulva and cervix has been found in university students with the use of Li consensus PCR technology (10). In Germany, a prevalence of 13–30% was found with the use of presumably less sensitive assay systems (2, 11). An HPV infection ascending from the cervix to the fallopian tube and ovary (including the epithelium) could hypothetically be one of the pathophysiological factors in the development of neoplasia of these tissues. HPV infection could, thereby, contribute to the inactivation of the p53 tumor suppressor gene previously found mutated in these cancers (12–15).

We have studied tumor cell lines and ovarian tumors from an unbiased sample of German Caucasian patients who had not been selected for known HPV risk factors, such as high average lifetime numbers of sexual partners. No association of advanced ovarian cancer with integrated HPV in the tumor cell genome was detected. If oncogenic HPVs were involved in initial transformation of the ovarian epithelial cell, viral DNA sequences may stay episomal (16) and may be lost before being carried on in the genome of the host in an integrated state during tumor progression (17). This may be an explanation of HPV being undetectable in advanced tumors and in passaged cells in vitro in this study. Theoretically, the selective pressure on HPV sequences could lead to retention of the E6 gene, although other regions of HPV including the L1 gene might be lost. This constellation was excluded in our study for the types 16 and 18, the most common forms of oncogenic HPV (18).

Adequate methodology and assay reliability are major concerns in molecular epidemiology. We have, therefore, applied an improved PCR-based test system that meets any applicable standard (19). Conflicting data have been reported from several studies regarding the association of HPV infection with neoplasms of the ovary. Each study tested a relatively small number of samples. HPV was detected in ovarian malignancies by a group in the United States in 10 of 12 (3) and by a group in Taiwan in 3 of 11 using an HPV 16/18 E6 gene PCR approach (7). The American group, however, retracted the paper, suspecting a contamination (5). Data from two other studies carried out in the United States could not detect HPV in ovarian cancer (4, 6).

In German Caucasians, common oncogenic HPV types do not appear to play a role in advanced epithelial ovarian cancer.

References


Human papillomavirus integration is not associated with advanced epithelial ovarian cancer in German patients.

I B Runnebaum, S Maier, X W Tong, et al.


Updated version
Access the most recent version of this article at:
[http://cebp.aacrjournals.org/content/4/5/573.citation](http://cebp.aacrjournals.org/content/4/5/573.citation)