RESEARCH NOTE

Detection of Type 16 Human Papillomavirus DNA in Formalin-fixed Invasive Squamous Cells from Laryngeal Cancers by Polymerase Chain Reaction

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To date approximately 77 distinct human papillomavirus (HPV) genotypes have been described and the genomic sequences of most of them have been fully analyzed. About 30 additional partial sequences have been obtained from putative novel HPV types, suggesting that the total number of existing HPV genotypes is in excess of 100 (H zur Hausen 1996 *Biochim Biophis Acta 1288*: 55-78). HPV cause benign tumors in the respiratory tract. Mounting evidence suggests that they also play a role in the etiology of a subset of head and neck cancers. Carcinomas in patients with a history of recurrent respiratory papillomatosis are caused clearly by persistent HPV interacting with one or more carcinogenic agents (BM Steinberg et al. 1996 *Cancer and Metastasis Reviews 15*: 91-112). Previous studies have suggested squamous neoplasia of the larynx and may also be HPV related (HAB Multhaupt et al. 1994 *Hum Pathol 12*: 1302-1305).

Current research also indicates that HPV may be involved in the development of benign tumors and also squamous cell cancers of head and neck (O Arndt 1994 *Laryngo-Rhino-Otol 73*: 527-532). The first reports of the presence of HPV in malignant tumors of the oral cavity appeared in 1985 (EM de Villiers et al. 1985 *Int J Cancer 36*: 575-578). They were followed by similar reports on HPV DNA in individual cases of cancer of the larynx. Although subsequently, by using polymerase chain reaction (PCR) analysis, the HPV detection data varied between 0 and 100% (zur Hausen *loc. cit*.).

Studies performed with reliable techniques commonly came up with positivity rates of between 10 to 20%. In these studies HPV16 was the most frequently encountered genotype (PJF Snijders et al. 1994 *J Gen Virol 75*: 2769-2775). Regularly HPV16 DNA has been found in invasive squamous cell cancers of the larynx in a relative high percentage (Arndt *loc. cit*.).

In the present study the presence of HPV16 in 40 formalin-fixed and paraffin-embedded tissues from laryngeal cancers, 20 glottic and 20 supraglottic, kindly supplied by The National Institute of Oncology and Radiobiology in Havana City, was investigated. The studied samples were selected from a group of patients aging from 34 to 58 years old who had presented a history of aggressive carcinoma of the larynx. All material was routinely fixed in 10% neutral-buffered formalin and paraffin-embedded. Sections were mounted on glass slides for histological diagnosis (Multhaupt *loc. cit*.) and the original histological diagnosis was confirmed.

DNA extraction from paraffin-embedded sections of laryngeal biopsies was performed by treatment of the sections (4-5 mm) with xylol and four cycles of ethanolic hydratation. After digestion with proteinase K solution and protein extraction with phenol choroform, DNA was precipitated (DK Shibata 1988 *J Exp Med 167*: 225-230).

HPV16 DNA was detected using a "Nested" PCR. PCR was performed using two specific modified primers to E6 region of HPV16. They are synthetic oligonucleotides, one of which is biotinylated while the second one is labeled with DNP hapten. Briefly, targed DNA in the sample was denatured and underwent a first amplification step with two specific non-modified primers. The product of this first amplification was diluted. Then a second amplification was carried on using the two specific modified primers. The product of this second amplification was immobilized on an Avidin coated tube. After fixation DNA duplexes were revealed by use of an anti-DNP monoclonal antibody conjugated to peroxidase (Amplicis HPV primers kit, Cis bio International, France).

In this study 20 patients (50%) with laryngeal cancer were HPV16 DNA positive. One retrospective study reviewed 36 tumor specimens, using PCR amplification of paraffin sections followed by south-

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ern blot hybridization, to test for the presence of HPV16 DNA. These workers found HPV16 in tumors from 45% of the cases (DM Fliss et al. 1994 *Laryngoscope 104*: 146-152). A number of studies have shown that laryngeal carcinomas do contain HPV16 DNA. The majority of them only examined a very small number of cases but 75% to 100% of the lesions studied were positive for HPV16 or HPV16 related DNA (Steinberg *loc. cit*.).

In the present study significantly more glottic cancers 14 of 20 (70%), were positive to HPV16 than supraglottic carcinomas because only 6 of 20 (30%) were positive to HPV16. Arndt (*loc. cit*.) studied a total of 100 formalin-fixed and paraffin-embedded laryngeal cancer specimens, 41 glottic and 59 supraglottic by use of E6 specific PCR for HPV16 DNA.

They detected HPV16 in 26 of 41 glottic cancers (63.4%) but only 10.2% (6 of 59) of the supraglottic carcinomas. These results are comparable to ours because we found HPV16 in 14 of 20 (70%) glottic cancers while only 6 of 20 (30%) of supraglottic cancers were positive for HPV16. There is a predominance of HPV16 DNA in supraglottic carcinomas but this could be explained because the level of some risk factors like nicotine and alcohol was significantly higher in the patients studied with supraglottic tumors. These patients had presented a history of alcohol consumption for more than five years and 100% were smokers since they were teenagers. The presence of both carcinogenic agents and HPV16 infection could be an important means by which to analyze the pathogenesis of laryngeal cancers.