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Case report

Human Papillomavirus type 11DNA in papillary squamous cell lung carcinoma

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Summary. We report a case of papillary squamous cell carcinoma of the lung developing in relation to a condylomatous papilloma and related to human papillomavirus (HPV) infection. The viral origin of the bronchial papillomatous lesion is strongly suggested by cytological and histological features with marked condylomatous changes. No viral capsid antigen was detected by immunohistochemistry. Transmission electron microscopy failed to reveal intranuclear viral-like particles in the papillary part of the carcinoma, but typical ultrastructural koilocytotic cells with irregular nucleus and coarse chromatin were observed. HPV DNA type 11 was detected by in situ hybridization using biotinylated probes on paraffin-embedded specimens, under stringent conditions (T_m −12°, 50% formamide). Papillary squamous cell carcinoma may result from the malignant conversion of benign squamous papilloma of the bronchus. HPV type 11 may be associated with malignant conversion of benign papilloma of the pulmonary tract, as in the upper respiratory tract. In situ hybridization with biotinylated probes is a relatively simple and appropriate method for retrospective analysis of HPV DNA sequences in surgical specimens.

Key words: Human papillomavirus – Primary lung carcinoma – In situ hybridization – Biotinylated probes

Introduction

The involvement of human papillomavirus (HPV) in upper respiratory tract tumours (benign papilloma and carcinoma of the larynx) has been well demonstrated by epidemiological (Bewtra et al. 1982), ultrastructural (Boyle et al. 1971) and immunohistochemical studies (Kashima et al. 1986) and by molecular hybridization with the Southern technique (Gissman et al. 1982; Mounts et al. 1982; Brandsma et al. 1986; Scheurlen et al. 1986; Kahn et al. 1986).

In the lower respiratory tract, the causative role of HPV has been suggested by morphological data: condylomatous-like lesions were described in about 30% of the bronchial mucosa next to invasive bronchial squarmous cell carcinomas (Syrjänen 1979), or inside well-differentiated squamous cell carcinomas (Syrjänen 1980a, b; Béjui-Thivolet et al. 1990) On electron microscopy, HPV-like particles were detected in a condylomatous solitary papilloma (Trillo and Guha 1988). Epidemiological studies reported malignant transformation of benign condylomatous papillomatosis (Rahman and Ziment 1983; Byrne et al. 1987).

Recently, HPV DNA sequences have been demonstrated by molecular DNA hybridization using in situ hybridization with a mixture of radioactive probes (Syrjänen et al. 1989a) and with biotinylated probes (Béjui-Thivolet et al. 1990) in primary lung squamous cell carcinomas.

We report the morphological features of a condylomatous bronchial papilloma with malignant conversion; we tested the presence of HPV infection signs by immunohistochemistry, ultrastructural study and by in situ hybridization with HPV DNA 6, 11, 16, 18 biotinylated probes on paraffin-embedded specimens.

Case report

The patient was a 45-year-old man, with pneumonitis. Chest roentgenogram showed a right upper pulmonary opacification. Fiberoptic bronchoscopy disclosed a 1-cm verrucous mass in the right upper lobe bronchus. On cytological examination of bronchial brushings, some koilocytotic cells were found. A biopsy specimen was taken from the polypoid lesion; the pathological diagnosis was solitary condylomatous papilloma.

Laryngoscopic examination was negative; there was no papilloma in the oral cavity, the nasopharynx, or larynx. The patient had a past history of heavy smoking (ten packs a year over 20 years). He reported no marked alcohol consumption. A right upper lobectomy was performed: the surgical specimen showed

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an endobronchial polypoid tumour in the right upper lobe bronchus with a 1.5×2 cm pulmonary invasion. Extensive acute bronchopneumonia was found, involving the right upper pulmonary lobe. The right hilar lymph nodes showed no metastatic squamous carcinoma. No primary or metastatic malignant neoplasm was found at any other sites. The postoperative course was uneventful.

Methods

Smears of bronchial brushings were fixed in 90% alcohol solution and were stained by the Papanicolaou method. Biopsy and surgical specimens of bronchial papilloma and lung tumour were fixed in neutral formalin and paraffin-embedded; all specimens were routinely examined after haematoxylin-eosin-safran (HES) staining.

For the detection and typing of HPV DNA by in situ hybridization two or three sections of the bronchial biopsy and the surgical specimens were tested with each HPV DNA biotinylated probe (types 6, 11, 16, 18) by in situ hybridization. Cloned HPV DNA types 6a, 11a, 16 and 18 (kindly provided by G. Orth, Paris and H. Zur Hausen, Heidelberg) were purified through caesium chloride gradient; plasmid DNAs were biotinylated using biotinylated 11 dUTP and a nick translation kit (Bethesda Research Laboratory). Paraffin-embedded sections were processed as described elswhere (Béjui-Thivolet et al. 1990).

For HPV typing high stringency conditions were used (T_m $-12°$ C, 50% formamide). The DNA-DNA hybrids were detected as reported earlier (Guérin-Reverchon et al. 1989) after successive incubations with antibiotin antiserum (Enzo New-York, NY), a biotinylated goat antirabbit antiserum (Enzo) and a streptavidin-alkaline phosphatase complex (BRL). Alkaline phosphatase was revealed after an incubation in a mixture of nitroblue tetrazolium and 5-bromo-4-chloro-3 indolyl phosphate (Blugene Kit, BRL). The slides were mounted in a glycerine-gelatin solution. Positive nuclei showed a purple precipitate under light microscopy.

Controls were performed on adjacent sections with biotinylated pBR 322 DNA or by omitting specific HPV DNA from the hybridization solution. Additional control slides of cells were used: CaSki cells containing 600 copies/cell of HPV DNA type 16 and HeLa cells containing 10–50 copies/cell of HPV DNA type 18 (Guérin-Reverchon et al. 1990).

To detect viral antigen indirect immunofluorescence was performed on paraffin-embedded sections with an HPV genus group specific rabbit antiserum prepared against sodium dodecyl sulphate (SDS)-dissociated purified HPV type 1.

Electron microscopy was performed on surgically excised specimens from condylomatous bronchial papilloma, after fixation in 4% glutaraldehyde in 0.2 M cacodylate buffer pH 7.4, postfixation in osmium tetroxide and dehydration in ethanol and embedding in araldite.

Results

Smears of bronchial brushing were stained by the Papanicolaou method. They contain many squamous cells, which are mainly single, and occasionally formed sheets. The cytoplasm is dense and glassy. Careful examination demonstrates perinuclear halos and degenerative vacuoles in some cells. Binucleation is common (Fig. 1). The nuclei are markedly enlarged and of variable appearance: some nuclei are dark and pyknotic while others are opaque and pale.

The initial bronchoscopic biopsy specimen stained by HES shows a typical pattern of squamous papilloma. The epithelium is arranged in branching fibrovascular cores, covered by a well-differentiated, stratified squamous epithelium. Some cells exhibit koilocytotic features: the nuclei are binucleated, hyperchromatic, and surrounded by a clear halo (Fig. 2).

Sections of the tumour of the right upper lobe bronchus show a wart-like endobronchial papilloma consisting of a well-formed connective tissue stroma covered with squamous metaplasic epithelium (Fig. 3). The upper cell layers display extensive koilocytotic changes characterized by binucleation and prominent perinuclear clearing. There are no malignant features in the hyperplastic squamous epithelium. The base of the solitary papilloma shows an unequivocal malignant transformation (Fig. 4). The bronchial wall and the adjacent pulmonary alveolar spaces are invaded by a well-differentiated squamous cell carcinoma. The middle part of the carcinoma contains a dense sheet of dyskeratotic superficial cells. Foci of tumour cells are koilocytotic. Histological diagnosis confirmed a well-differentiated squamous cell carcinoma arising on a condylomatous papilloma.

HPV type 11 was detected on the surgical specimen of the papillary part of the squamous cell carcinoma. The signal was always localized to the superficial layers, as shown in a low-power magnification (Fig. 5). At high magnification the staining was always nuclear, usually in foci of infected cells (Fig. 6). No HPV DNA was detected in the invasive part of the dyskeratotic carcinomatous cells. With HPV types 6, 16, 18, the reactions were negative.

Further controls on adjacent pathological tissue sections without DNA probes or with pBR 322 plasmid DNA were negative. There was no nuclear staining in the normal bronchial columnar mucosa or in the pulmonary alveolar tissue with any HPV DNA probe or with pBR 322 plasmid DNA. Positive controls were included. The nuclei of CaSki cells were positive only with HPV 16 probe, whereas nuclei of HeLa cells were positive only with HPV 18. Types 6 and 11 HPV were detected in other condylomatous tissue specimens of genital origin.

No viral antigen was detectable by indirect immunofluorescence in the bronchial papilloma or in the pulmonary tumour.

Electron microscope fields selected on semi-thin sections with koilocytotic cells showed ultrastructural features of mature squamous cells with well-preserved desmosomes and cell junction structures. In such epithelium rare koilocytotic cells are found; they are characterized by an irregular nucleus with coarse chromatin and a perinuclear zone containing sparse ribosomes. No viral particles are seen.

Discussion

In a condylomatous bronchial papilloma associated with a squamous cell carcinoma we found HPV DNA type 11 by in situ hybridization using biotinylated probes on paraffin-embedded specimens.

Papillary tumours of the bronchus comprise a group of tumours of different degrees of malignancy ranging from the benign squamous papilloma to the pulmonary

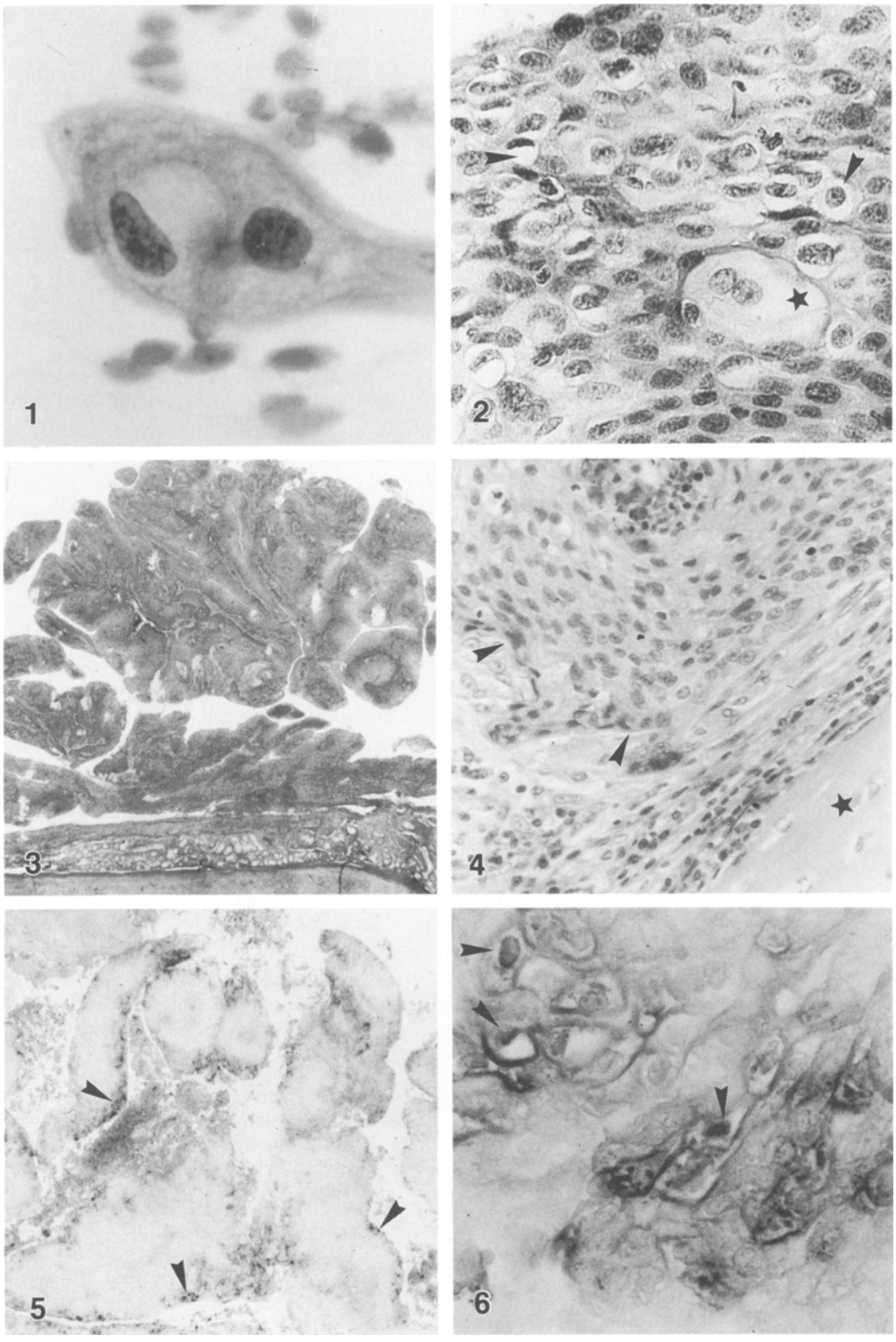

Fig. 1. Koilocytotic-like cell: binucleated squamous metaplastic cell with perinuclear halo and degenerative vacuole. Bronchial brushing, Papanicolaou, × 1250

Fig. 2. Bronchial papilloma: squamous metaplastic epithelium with koilocytotic cell(*) and numerous cells with perinuclear halos (arrows). Bronchial biopsy, HES, × 500

Fig. 3. Condylomatous endobronchial papilloma. Right upper lobectomy – HES, × 30

Fig. 4. Base of the solitary papilloma shows evident malignant transformation (*arrows*) with bronchial wall (*) invasion. HES, × 320

Fig. 5. Same lesion as in Fig. 3. Numerous positive nuclei in the outer layers (*arrows*). In situ hybridization with HPV DNA 11 probe, × 60

Fig. 6. Same lesion as in Figs. 3 and 5. Positive nuclei in the koilocytotic cells (*arrows*). In situ hybridization with HPV DNA 11 probe, × 480

squamous cells carcinoma. Solitary bronchial papilloma is a very rare disease which usually occurs in adults. Approximately 50% of the solitary adult tumours become malignant (Al Saleem et al. 1968; Mounts et al. 1982). It is best to reserve the diagnosis of carcinoma for those cases with unequivocal pulmonary invasion, as in our case. The bronchial papillomatous lesion exhibits prominent cytological and histological condylomatous changes. Similarities between solitary squamous papilloma of the bronchus and uterine cervix condylomas have been reported (Moore and Lattes 1959; Maxwell et al. 1985). Only one study (Rubel and Reynolds 1979) has described the cytological features from squamous papilloma on bronchial washing in common with cervical condyloma.

Our data report on bronchial brushings of a polypoid bronchial tumour with the finding of typical koilocytotic cells. Histological features with well-formed fibrovascular stroma lined with squamous metaplasic epithelium are similar to condylomata acuminata as previously reported by Trillo and Guha (1988). These changes were also observed in primary squamous cell carcinoma. On histological specimens, condylomatous-like nuclear modifications are usually seen in 30% of the bronchial mucosa adjacent to pulmonary carcinomas (Syrjänen 1979). Recently, we described, on routine staining, typical condylomatous-like changes in 12 of 33 (36%) primary well-differentiated squamous cell carcinomas (Béjui-Thivolet et al. 1990), as previously reported in two other series (Syrjänen 1980a, b). All these morphological findings suggest that primary lung squamous cell carcinomas are related to HPV infection. Intranuclear HPV-like particles were demonstrated by an ultrastructural study in solitary condylomatous papilloma of the bronchus (Trillo and Guha 1988). We, like others (Bewtra et al. 1982) have failed to reveal HPV-like particles. Few ultrastructural studies have been carried out since this method is insensitive, expensive, and time consuming. Immunochemistry permits identification of the common capsid antigen of HPV in some laryngeal and bronchial papillomas (Mounts et al. 1982; Trillo and Guha 1988). However, HPV-infected tissues showing high degrees of malignancy, like laryngeal carcinomas, rarely contain HPV antigen (Brandsma et al. 1986; Kahn et al. 1986).

In situ hybridization applied to fixed tissues offers the advantage of retrospective comparison between HPV typing and histopathological lesions on adjacent sections. Our technique permitted the localization of HPV DNA in the koilocytotic cells of the endobronchial papillary tumour. In situ methods are sensitive even after fixation when the copy number of DNA per cell is relatively high (Syrjänen et al. 1989a; Béjui-Thivolet et al. 1990). In our study no viral sequence was detected by in situ hybridization in carcinomatous cells of the pulmonary squamous cell carcinoma; a possible explanation is the low copy number of HPV DNA in these cells. It is probably at the borderline of the sensitivity of detection by in situ hybridization (Cornelissen et al. 1989). However, as the HPV DNA is preferentially located in foci of infected cells in lung carcinomas (Béjui-Thivolet et al. 1990), we may suppose that some sections were outside the foci.

Previous investigations have reported the presence of potentially oncogenic HPV types 16/18 or benign HPV types 6/11 by in situ hybridization with a cocktail of radioactive probes in primary squamous cell carcinoma (Syrjänen et al. 1989a) or with biotinylated probes (Béjui-Thivolet et al. 1990). HPV types 16/18 have been detected more frequently than the others. HPV DNA type 11 has already been detected in a primary lung carcinoma with in situ hybridization using biotinylated probes under stringent conditions (Béjui-Thivolet et al. 1990). HPV DNA type 11 has also been demonstrated with the Southern blot method in a laryngotracheobronchial papillomatosis and metastatic squamous cell carcinoma of the lung (Byrne et al. 1987).

The papillary squamous cell carcinoma may result from the malignant conversion of the endobronchial squamous papilloma characterized by typical condylomatous features and HPV DNA type 11. This suggests that HPV DNA 11 might be involved in the process of malignancy. Cases of malignant transformation associated with HPV 11 have previously been reported in condylomas from other sites, such as larynx or uterine cervix (Syrjänen 1989b; Wells 1987).

It has been suggested (Syrjänen 1979) that HPV would be able to induce benign or malignant epithelial proliferation only in metaplastic squamous bronchial epithelium. The possibility of synergistic mechanisms between HPV and chemical or physical carcinogens such as cigarette smoking and radiotherapy may be considered (Zur Hausen 1982); HPV may act as a tumour promotor.

In conclusion, the case of papillary squamous cell carcinoma reported here illustrates a probable role for HPV 11 in the process of malignant conversion in primary lung papillary carcinoma. The initial bronchial papilloma, which is a very rare lesion, is characterized by typical condylomatous changes and contains HPV DNA type 11 in the nuclei of koïlocytotic cells. In situ hybridization with biotinylated probes permits the retrospective evaluation of routinely processed material.

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