

Demonstration of Papillomavirus Structural Antigen in Human Urinary Bladder Neoplasia

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Summary—Clear cells showing characteristic features of koilocytotic atypia were found in 41 (82%) of 50 paraffin sections of urinary bladder tumour. Papillomavirus common structural antigen was detected in seven (14%) of the samples using an avidin-biotin peroxidase staining technique. The results suggest a role for the human papillomavirus in the aetiology of urinary bladder neoplasia.

Transitional cell carcinoma of the urinary bladder is common, though its aetiology is not completely understood. The bladder is particularly vulnerable to environmental carcinogens and many factors have been identified as probable causes of bladder cancer (Hoover and Strasser, 1980; Morrison, 1984; Vaught and King, 1984; Gonzalez *et al.*, 1985; Hartge *et al.*, 1985).

Papillomaviruses have been implicated in several animal and human cancers (Zur Hausen, 1977; Jarrett, 1981). The most convincing case for association of the human papillomavirus (PV) with human malignancy has been established for genital cancer (Zur Hausen, 1982). PV common structural antigen has been demonstrated in both genital warts (condylomata acuminata) and cervical intra-epithelial neoplasia (CIN) (Woodruff *et al.*, 1980; Morin *et al.*, 1981; Kadish *et al.*, 1986). In addition, human PV DNA sequences have been detected in 96% of precancerous cervical lesions (Kadish *et al.*, 1986). Condylomas, which are benign lesions commonly affecting the perianal skin and the external genitalia, are also less commonly found at deeper mucosal sites such as the urethra and urinary bladder (Kleiman and Lancaster, 1962; Nielsen, 1975; Pettersson *et al.*, 1976; Murphy *et al.*, 1983; Syrjanen and Pyrhonen, 1983; Keating *et al.*, 1985; Libby *et al.*, 1985).

Urethral condylomas have been shown to contain human PV structural antigens (Murphy *et al.*, 1983;

Syrjanen and Pyrhonen, 1983). Tumours of the urinary bladder of cattle have also been associated with PV (Olson *et al.*, 1959, 1962, 1965; Brobst and Olson, 1965).

The possible role of human PV in urinary bladder neoplasia was investigated firstly by examining biopsies histologically for the characteristics of koilocytotic atypia, a feature associated with PV infection, and secondly by staining for the presence of PV structural antigen using an avidin-biotin peroxidase technique (Rabbit IgG Vectastain ABC Kit, Vector Laboratories Inc.) and an antibody to PV common structural antigen (Rabbit anti-bovine PV, Dako).

Materials and Methods

Transurethrally resected urinary bladder tumours from 50 patients were studied. The patients ranged in age from 49 to 89 years with a male:female ratio of 4:1.

Paraffin sections stained by haematoxylin and eosin were examined microscopically. PV common structural antigen was detected as follows.

Paraffin sections of formalin-fixed samples were deparaffinised in xylene, hydrated in alcohol, rinsed in distilled water, washed in Tris-buffered saline (TBS) pH 7.6, and incubated in 0.5% hydrogen peroxide in methanol for 10 min to remove endogenous peroxidase. Following a brief wash in TBS, the sections were incubated in dilute normal rabbit serum for 20 min. The excess serum was

blotted from the sections and the slides then re-incubated in rabbit anti-bovine papillomavirus (BPV-1) diluted 1:80 with TBS for a further 20 min. After a brief wash in TBS, the sections were incubated in biotinylated anti-rabbit immunoglobulin solution for 30 min. The sections were again rinsed briefly in TBS and finally incubated in Vectastain ABC reagent for 60 min. Following a brief wash in TBS, the peroxidase was developed for 5 to 10 min in 0.05 g/dl 3'3'diaminobenzidine in TBS with 0.1 cm³ 1% hydrogen peroxide added prior to use. The sections were then dehydrated in alcohol, cleared in xylene and coverslipped without counterstaining and examined microscopically. The specificity of the immunohistochemical staining was confirmed by the replacement of the rabbit anti BPV-1 by normal rabbit serum. A histologically active, benign viral wart was used as control.

The technique was also performed on 40 colposcopy biopsies showing histological evidence of koilocytosis.

Results

Of the 50 bladder tumours sampled, 31 (62%) were histologically reported as papillary transitional cell carcinoma, and 18 (36%) were of the solid type. The remaining sample consisted of necrotic material only. Microscopic examination of stained sections demonstrated the presence of cells having the characteristic features of koilocytotic atypia (perinuclear clear zone, peripheral cytoplasmic condensation and hyperchromatic nuclei) in 30 (97%) of the papillary and 11 (61%) in the adjacent urothelium of the solid type (Fig. 1A). These cells closely resembled the koilocytes seen in a cervical lesion (Fig. 1B).

Immunohistochemical staining of bladder tumour sections with antibody to PV common structural antigen using an avidin-biotin peroxidase technique produced dark brown nuclear staining in seven (14%) of the samples studied. Positively stained cells with characteristic features of koilocytotic atypia were noted in the urothelium adjoining the solid tumours but not in the tumours themselves (Fig. 2). The papillary tumours revealed scattered positive nuclei (Fig. 3). The characteristics of the bladder tumours are summarised in Table 1.

By the same technique, 11 (27.5%) of 40 colposcopy biopsies showing histological features of koilocytotic atypia were found to have nuclei positive for PV structural antigen (Table 2).

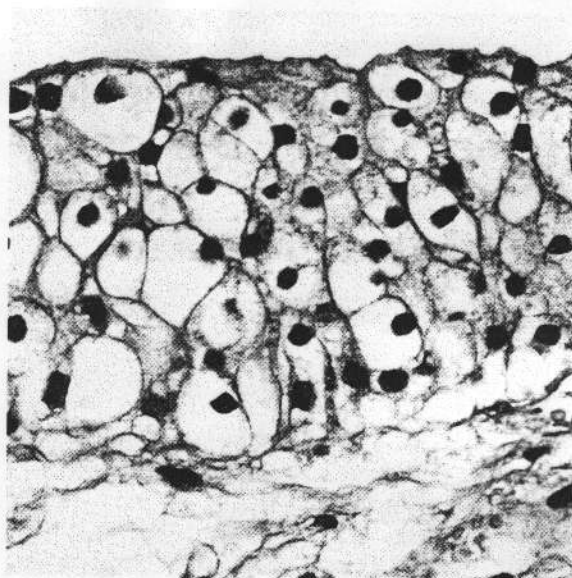


Fig. 1A Areas resembling koilocytotic atypia in urothelium adjacent to a solid transitional cell carcinoma. (H and E \times 660).

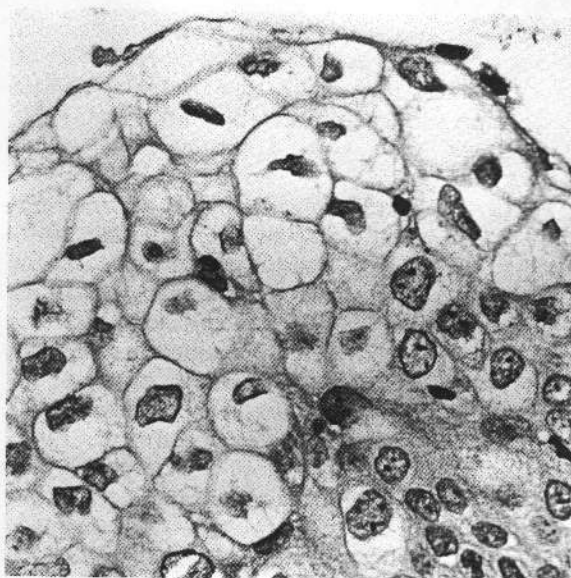


Fig. 1B Cervical colposcopy biopsy showing areas of koilocytotic atypia. (H and E \times 660).

Discussion

Papillomaviruses infect many higher vertebrates, including man. They are host and tissue specific and produce benign epithelial tumours which, although they generally regress, may undergo malignant transformation (Jenson *et al.*, 1984; Zur

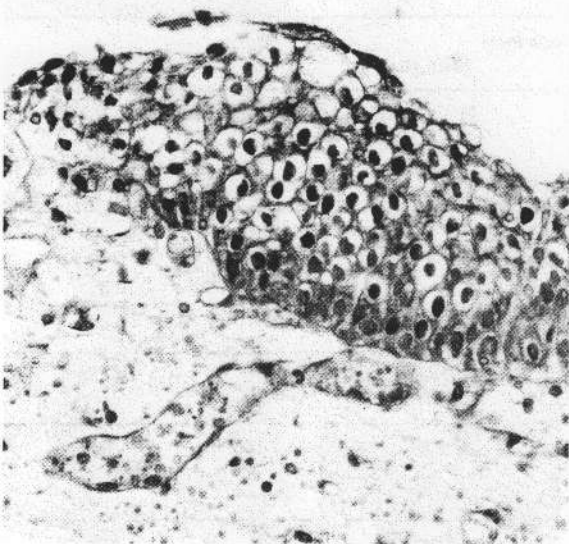


Fig. 2A Urothelium showing nuclear positivity for PV structural antigen adjacent to a solid transitional cell carcinoma. Note the positive reaction of stromal mast cell granules due to their affinity for conjugated avidin (Tharp *et al.*, 1985). (Avidin-biotin peroxidase $\times 330$).

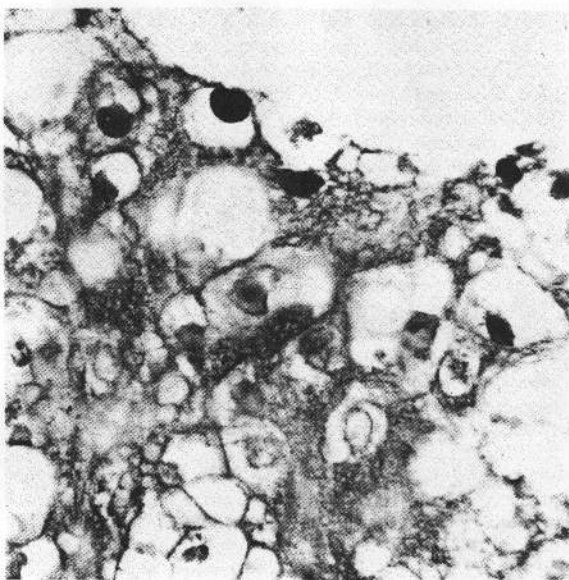


Fig. 2B Urothelium adjacent to a solid transitional cell carcinoma showing nuclear positivity for PV structural antigen in cells showing characteristics of koilocytotic atypia. (Avidin-biotin peroxidase $\times 660$).

Hausen *et al.*, 1984). PV common structural antigens have recently been detected in lesions from various anatomical sites (Table 3).

PV infections of the uterine cervix are common and determine the incidence of koilocytes in both cervical smears and biopsies (Fletcher, 1983). The koilocyte is the result of a process characterised by cellular changes due to the cytopathogenic effect of the human PV. The cells involved show perinuclear clear zones with small, sometimes irregular hyperchromatic nuclei and peripheral cytoplasmic condensation (Casas-Cordero *et al.*, 1981; Recher and Srebnik, 1981).

The clear appearance of the cytoplasm is also a characteristic of cells of normal and papillary transitional epithelium. Although these clear cells are often absent in solid transitional cell carcinomas (Murphy, 1983), this study has shown the presence of cytoplasmic vacuolisation and peripheral cytoplasmic condensation suggestive of koilocytotic atypia in the urothelium adjacent to these tumours. PV antigen was also demonstrated in some of the nuclei of these cells.

PV antigen has been detected in 16 to 50% of cases of cervical intra-epithelial neoplasia (Table 3). However, recent work has shown that 96% of such tumours contain PV DNA (Kadish *et al.*, 1986). Therefore immunological detection of PV antigen may not provide the best estimate of the extent of involvement of human PV in bladder neoplasia. Nonetheless, our results show the pres-

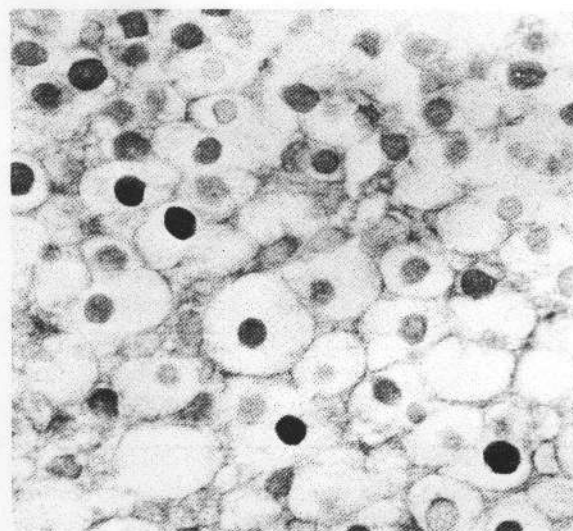


Fig. 3 Papillary transitional cell carcinoma showing nuclei positive for PV structural antigen. (Avidin-biotin peroxidase $\times 660$).

Table 1 Characteristics of Urinary Bladder Tumour Samples

	Histopathology						Totals 50*
	Papillary grading			Solid grading			
	I	II	III	II	III	IV	
Number sampled	13	13	5	2	10	6	
Number positive for PV structural antigen	1	2	1	1	2	0	7

* Includes one sample consisting of necrotic material only.

Table 2 Characteristics of Colposcopy Biopsies showing Histological Evidence of Koilocytotic Atypia

	Benign	Histopathology grading			Totals
		CIN I	CIN II	CIN III	
Number sampled	15	7	10	8	40
Number positive for PV structural antigen	2	3	2	4	11

Table 3 Frequency of Detection of Papillomavirus Structural Antigen in Various Lesions

Lesion	Frequency (%)	Reference
Plantar warts	100	Lucia <i>et al.</i> (1984)
<i>Verruca vulgaris</i>	66	Lucia <i>et al.</i> (1984)
	75	Penneys <i>et al.</i> (1984)
Cutaneous papillomas	50	Braun <i>et al.</i> (1983)
Cutaneous dysplasias	5	Braun <i>et al.</i> (1983)
Oral papillomas	83	Loning <i>et al.</i> (1984)
Oral leukoplakias	40	Loning <i>et al.</i> (1984)
Oesophageal papillomas and hyperplasias	31	Winkler <i>et al.</i> (1985)
Adult laryngeal papillomas	0	Costa <i>et al.</i> (1981)
Juvenile laryngeal papillomas	0	Lucia <i>et al.</i> (1984)
	50	Costa <i>et al.</i> (1981)
Urethral papillomas	44	Dean <i>et al.</i> (1983)
Urethral condylomas	62	Murphy <i>et al.</i> (1983)
Genital condylomas	10	Lucia <i>et al.</i> (1984)
	62	Gupta <i>et al.</i> (1983)
Cervical atypia (colposcopy)	16	Singer <i>et al.</i> (1985)
	20	Walker <i>et al.</i> (1983); Dyson <i>et al.</i> (1984); Singer <i>et al.</i> (1985)
	23	Singer <i>et al.</i> (1985)
	27	McCance <i>et al.</i> (1983)
	30	Singer <i>et al.</i> (1985)
	50	Kadish <i>et al.</i> (1986)

ence of human PV antigen in a significant number of bladder tumour samples and make the determination of the presence of human PV DNA sequences in these tumours of great interest. These studies are now in progress.

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