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Malignant Inverted Papilloma of the Urinary Bladder : The Histopathological Aspect of Malignant Potential of Inverted Papilloma

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To investigate the histopathological characteristics of inverted papillomas of the urinary bladder, including the possibility of malignant transformation, we studied the indicators of cellular proliferation activity in 7 inverted papillomas of the bladder including two cases of malignant inverted papilloma of the bladder. PCNA expression rates in two cases of malignant inverted papilloma were higher than in benign inverted papillomas. Mean numbers of AgNORs per nucleus in malignant inverted papillomas were much more than in benign inverted papillomas. The c-erbB-2 oncoprotein was expressed only in malignant inverted papillomas. These results suggest that PCNA expression rate, mean number of AgNORs per nucleus and c-erB-2 oncoprotein expression may be merited as good indicators to detect the inverted papilloma with more proliferative and aggressive lesions, and with the potential of malignant transformation.

Key Words : *Inverted papilloma, Urinary Bladder, Malignant transformation*

INTRODUCTION

Inverted papillomas are rare tumors of the lower urinary tract, characterized by an endophytic growth pattern and generally regarded as benign urothelial lesions. This type of tumor, which is now recognized as an independent entity, was first described in detail in 1963 by Potts and Hirst (1963). Since then, a number of cases have been reported in the urinary bladder (Cumminings, 1974). Although inverted papillomas are usually considered benign, recurrence was reported in a histologically benign case (DeMeester et al., 1975). These lesions have been reported to demonstrate the potential for malignant transformation (Lazarevic et al., 1978 ; Uyama et al., 1980) and recently reported cases of inverted papilloma of the bladder, renal pelvis and ureter demonstrated the histologic characteristics of malignancy (Whitesel, 1982 ; Uyama and Moriwaki, 1981). Concurrence with carcinoma in situ has also been reported (Altaffer et al., 1982).

We report two cases of malignant inverted papilloma of the urinary bladder. And, to investigate the histopathological characteristics of inverted papilloma of the bladder including the potential of malignant transformation of these tumors, we studied the established indicators of cellular proliferation activity such as proliferating cell nuclear antigen (PCNA) expression rate, mean numbers of silver-stained nucleolar organizer regions (AgNORs) per nucleus and expression

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of c-erbB-2 oncoprotein in 7 inverted papilloma including two cases of malignant inverted papillomas of the bladder.

MATERIALS AND METHODS

Included in this study were 5 benign inverted papillomas, one malignant transformation from benign inverted papilloma and one malignant inverted papilloma tissues of the urinary bladder from the files of the Department of Urology and Pathology, Korea University Hospital, from January 1988 to June 1993. All specimens had been removed by transurethral resection of the bladder lesion. The tissues were routinely formalin fixed, paraffin embedded and stained with hematoxylin and eosin (H & E).

Immunohistochemical staining of PCNA

LSAB immunoperoxydase method was used for staining of PCNA in routinely formalin-fixed and paraffin-embedded tissue sections (Dierendonck et al., 1991) by using 1 : 10 monoclonal mouse antibody, DAKO-PCNA, PC-10 (DAKO, USA). All slides were reviewed independently by two investigators (Cheon and Kim). Three adenocarcinomas of the prostate tissues were used as a positive control and substitution of the primary antibody with an irrelevant negative control reagent as a negative control.

The pattern of PCNA expression was classified into four scores by estimating the percentage of stained tumor cell nuclei : score 0 (negative) was defined the tumor contained less than 5% of positive nuclei ; score 1 was the tumor showed patchy positive nuclei ranging from 5 to 19% ; The tumor of score 2 showed heterogeneously positive nuclei ranging from 20 to 75% ; score 3 was homogeneously positive for PCNA in more than 75%.

Silver colloid staining for nucleolar organizer regions (AgNORs)

All samples were stained with colloidal silver nitrate solution and numerated the positive dots in the nuclei (Giri et al., 1989). Careful focusing allowed the AgNORs to be visualized as black dots arranged both in clusters and clumps and as individual "satellites" within the cell nucleus. To assess the reproducibility of the counting procedure, all counts were repeated. The repeated counts for each area varied by less than 10% of the initial value.

Immunohistochemical staining of c-erbB-2 oncoprotein

Immunohistochemistry was carried out using the indirect immunoperoxidase technique as previously described (Wright et al., 1989) by using polyclonal rabbit anti-human immunoglobulin (Dakopatts, Copenhagen, Denmark).

The positive reaction was defined when distinct cell membrane staining was observed, although a faint cytoplasmic staining could be noted. Staining results were expressed as absent, weak, moderate and strong.

RESULTS

Clinical and histopathological characteristics

Inverted papilloms were cystoscopically polypoid (pedunculated or sessile) and often appeared as nodular, gray-white surface elevations. They were usually 1 to 2 cm in diameter. Six of inverted papilloma were situated within the trigone and neck of the bladder. None of them had been recognized as inverted papilloma before transurethral resection of the bladder lesions. At the time of diagnosis the average age of patients was 52 years(range, 37-63 years). The patients with malignant inverted papillomas aged 47 and 54 years. Occurence predominated in the male with a male-to- female ratio of 5 : 2. The patients with malignant inverted papilloma were male.

Benign inverted papilloma

In five patients with benign inverted papilloma there were peculiar microscopic configurations. The epithelial cord pattern was invaginated deeply into the body of the lumen, while the external epithelial surface is smooth and without fronds. The lesion was usually covered (at least in part) by a thin epithelial layer and the epithelial cells were identical with normal transitional epithelium. They had little or no mitotic activity and cellular atypia was minimal (Fig. 1A). Among these five benign inverted papillomas one occurred separately with transitional cell carcinoma of the bladder.

Five patients diagnosed with benign inverted papilloma have been closely followed-up to recent days with periodic cystoscopy and urine cytology, and there is no evidence of tumor recurrence or development of new lesion of the bladder.

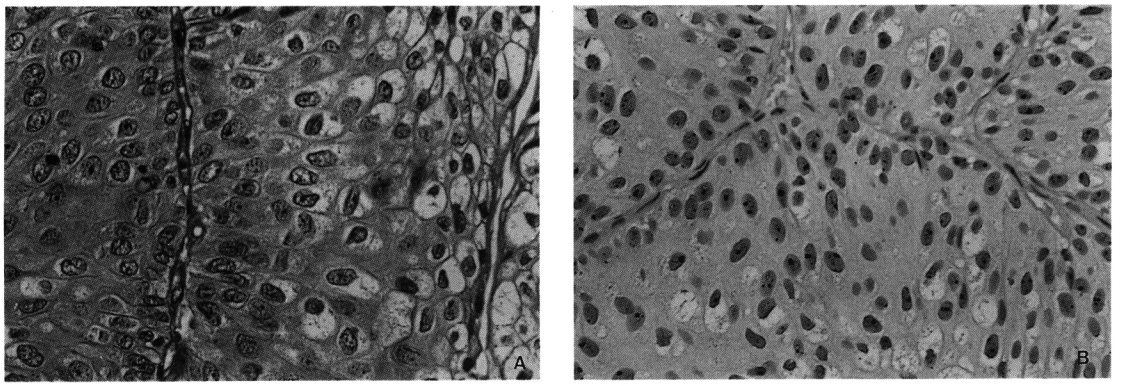

Fig. 1. Inverted papilloma. A, The higher magnification shows slight nuclear atypical features and rare mitoses. Most tumor cells have round to ovoid nuclei with one or two nucleoli and moderately abundant cytoplasm (×400). B, Siver-stained NORs : AgNORs are relatively round, regular, and similar from cell to cell (×400).

Malignant transformation and Malignant inverted papilloma

In two other patients the second patterns of inverted papillomas exhibited increased cellularities, nuclear crowdings and some dispolarities ; Furthermore, the tumor that was approximately 11-13 cells thick, markedly increased above the 6-8 cell thickness of normal urothelium (Fig. 2A-C & 3A-C). These were interpreted as malignant transformation of the surface urothelial component of an benign inverted papilloma (Fig. 2), and malignant inverted papilloma (Fig. 3), or transitional cell carcinoma with a feature of inverted papilloma. The tumor interpreted as malignant transformation had concomitant benign inverted papilloma in a same specimen (Fig. 2).

A 54-year-old man with malignant inverted papilloma transformated from benign inverted papilloma had been treated with left nephroureterectomy and bladder cuff resection 33 months ago, when there was no evidence of metastatic lesion on radiologic and pathological evaluation. Thereafter he had been well for 24 months. When follow-up cystoscopy revealed multiple papillary masses on the neck and posterior wall of the urinary bladder, and there were multiple metastatic lesions on chest P-A. After complete transurethral resection of the bladder tumor (TURBT) and four cycles of MVAC systemic chemotherapy, the size of metastatic lesions on both lungs were markedly reduced. The patient tolerated well during systemic chemotherapy and has been followed-up for six months after TURBT.

The second patient of 47-year-old man with malignant inverted papilloma, grade 2, received single six weeks course of BCG intravesical immunotherapy after TURBT, and there has been no evidence of tumor recurrence or progression during 52 months of follow-up.

PCNA expression

There was a distinct difference of PCNA expression rate on the basis of their patterns of tumor cell staining between benign and malignant inverted papilloma. Five cases benign inverted papilloma shonwed either negative (Score 0, n1) or patchy (score 1, n4) nuclear immunoreactivities, while two malignant inverted papillomas showed inhomogeneous (score 2) and homogeneous (score 3) nuclear immunoreactivities (Fig. 2D & 3F). The nuclei of two malignant inverted papillomas exhibited higher PCNA expression rate than benign inverted papilloma of the bladder (Table 1).

Mean numbers of AgNORs per nucleus

The mean numbers of AgNORs per nucleus for five benign inverted papillomas were 1.5, 2.2, 2.2, 2.5 and 2.7 (average 2.22)respectively (Fig. 1B). Multiple AgNORs were present in two malignant inverted papillomas with mean numbers of AgNORs per nucleus of 3.6 and 3.8 (Fig. 3D). The AgNORs were round, regular and uniform in benign inverted papillomas ; additionally, larger nucleoli and variation in size of AgNORs were visualized in the nuclei of the

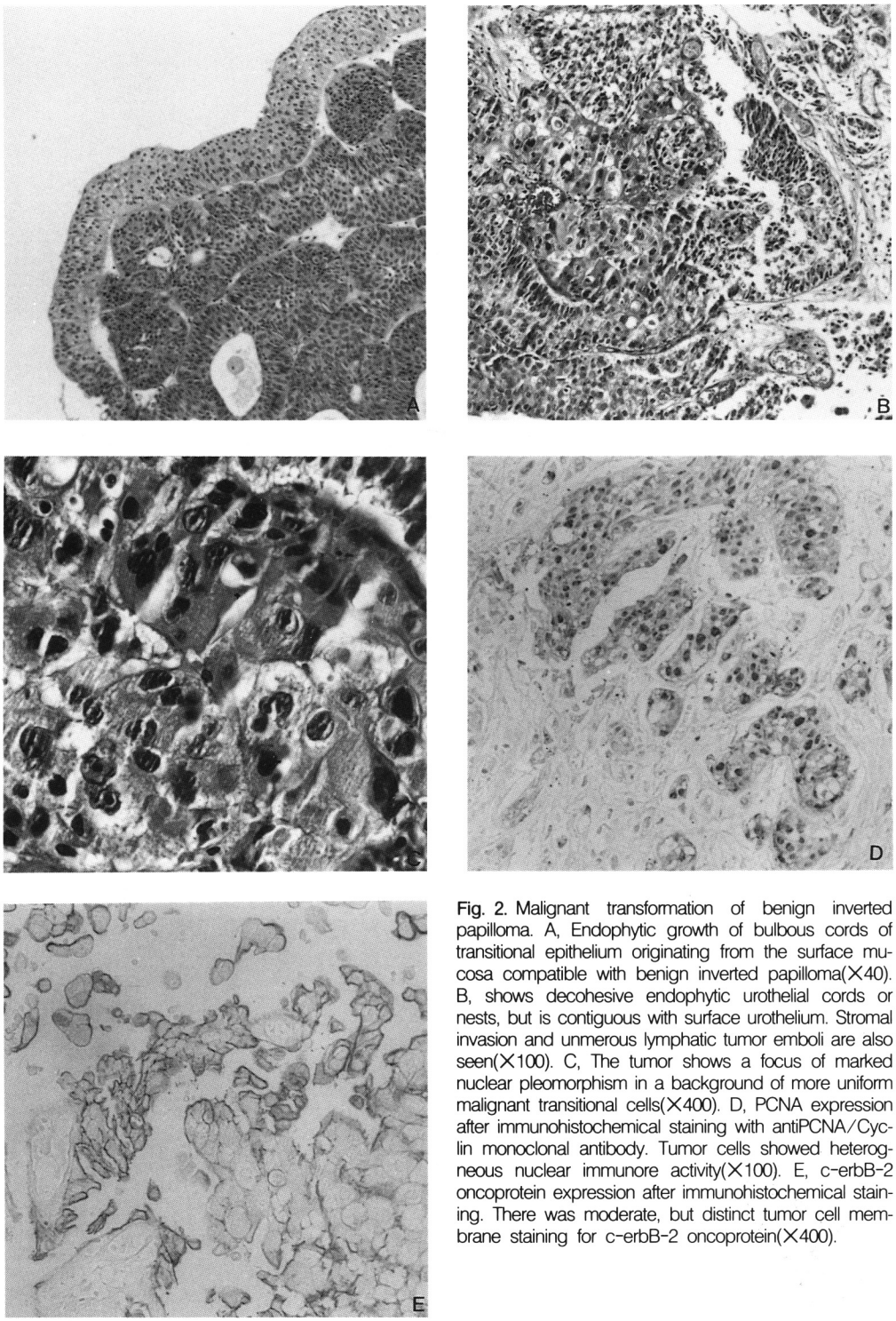

Fig. 2. Malignant transformation of benign inverted papilloma. A, Endophytic growth of bulbous cords of transitional epithelium originating from the surface mucosa compatible with benign inverted papilloma(×40). B, shows decohesive endophytic urothelial cords or nests, but is contiguous with surface urothelium. Stromal invasion and unmerous lymphatic tumor emboli are also seen(×100). C, The tumor shows a focus of marked nuclear pleomorphism in a background of more uniform malignant transitional cells(×400). D, PCNA expression after immunohistochemical staining with antiPCNA/Cyclin monoclonal antibody. Tumor cells showed heterogneous nuclear immunore activity(×100). E, c-erbB-2 oncoprotein expression after immunohistochemical staining. There was moderate, but distinct tumor cell membrane staining for c-erbB-2 oncoprotein(×400).

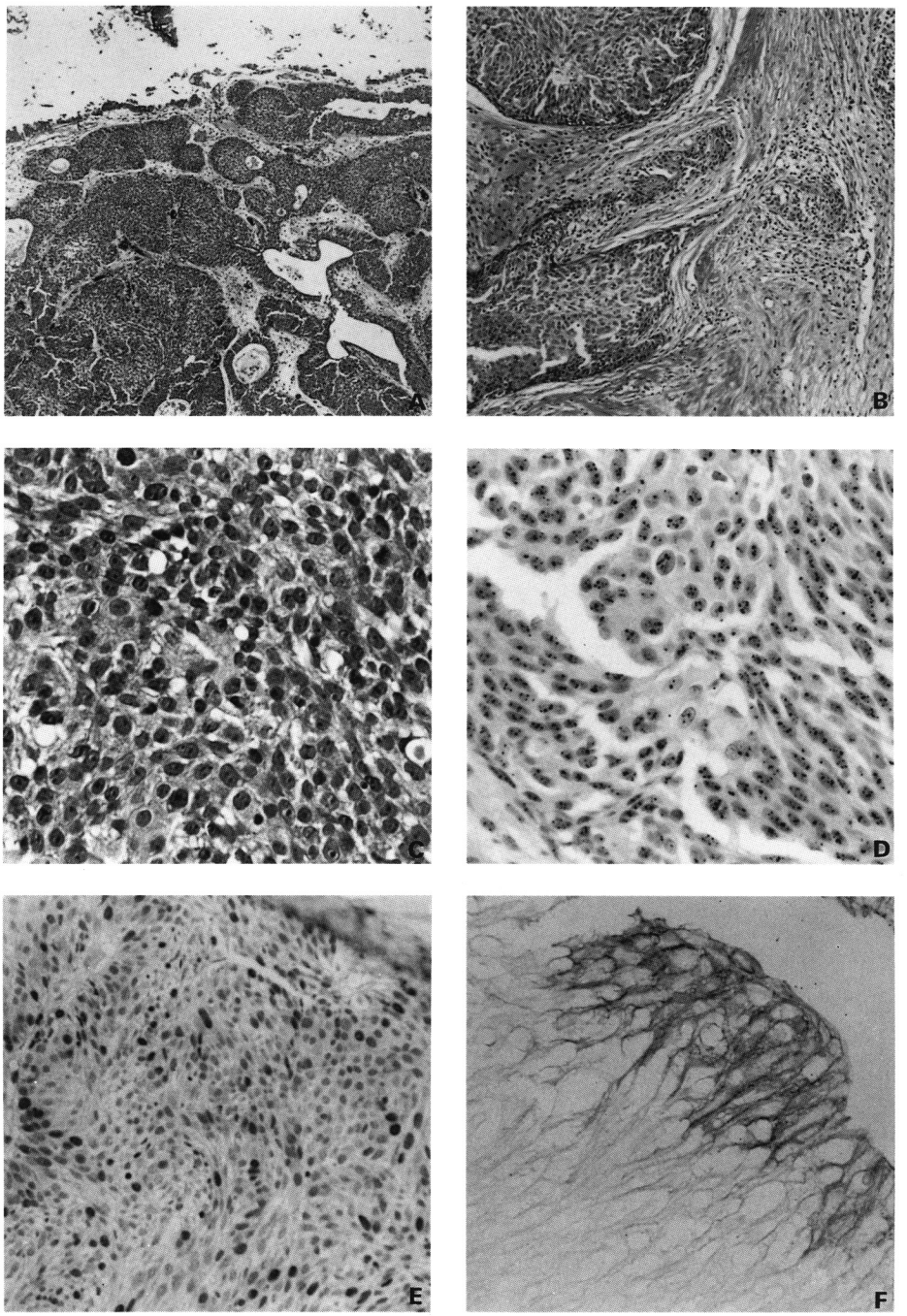

Fig. 3. Malignant inverted papilloma. grade 2. A, Endophytic growth of irregular complex urothelial cords contiguous with surface epithelium, which is histologically unremarkable(×400). B, shows invasive growth pattern to stroma(×100). C, Higher magnification shows anisonucleosis and more compact hyperchromatic cells with two or more prominent nucleoli and irregular AgNORs(×400). D, Silver-stained NORs : The tumor cell nuclei contain multiple and irregular AgNORs(×400). E, PCNA expression after immunohistochemical staining after anti-PCNA/Cyclin monoclonal antibody. Tumor cells showed homogeneous nuclear immuno-reactivity(×200). F, c-erbB-2 oncoprotein expression after immunohistochemical staining. There was distinct and strong tumor cell membrane staining for c-erbB-2 oncoprotein(×400).

Table 1. Results of immunohistochemical staining of PCNA, c-erbB-2 oncoprotein and silver-staining for nucleolar organizer regions(AgNORs)

Case No.	Inverted Papilloma	PCNA expression (score*)	c-erbB-2 expression	AgNORs count
1	benign	0	negative	1.5
2	benign	1	negative	2.2
3	benign	1	negative	2.2
4	benign	1	negative	2.5
5	benign	1	negative	2.7
6	malignant**	2	moderate	3.6
7	malignant	3	strong	3.8

*Score 0 ; negative(<5%)
1 ; patchy(5-19%)
2 ; inhomogeneous(20-75%)
3 ; homogeneous(>75%)
**The case of malignant transformation from benign inverted papilloma

malignant inverted papillomas. Two malignant inverted papillomas demonstated greater increas mean AgNORs counts than benign inverted papilloma of the bladder.

c-erbB-2 oncoprotein expression

c-erbB-2 immunohistochemical membrane staining was not seen in five benign inverted papillomas. One inverted papilloma demonstrated a diffuse, faint intracytoplasmic staining pattern, but without coexistence specific outlining of the cell membranes. Consequently, this was not classified as c-erbB-2 immunoreactivity. In contrast, both malignant inverted papillomas exhibited high degrees (moderate and strong) of c-erbB-2 immunocreactivity (Fig. 2E & 3F). There was c-erbB-2 immunoreactivity only in malignant inverted papillomas of the bladder.

DISCUSSION

Inverted papilloma is an uncommon benign tumor of the urinary tract. Grossly this tumor may appear as a papilloma but more typically it is a sessile or pedunculated polyp with smooth or lobular gray or white surface. The tumors are typically solitary although rarely they are multiple. They are usually 1 to 2 cm in diameter but may be larger. Bulbous projections may convey the gross impression of a papillary tumor, and they may result in a radiologic filling defect.

Because of the distinctive histologic features and the specific cell differentiation, it seems justified to distinguish between two basic types of inverted papillomas which we termed trabecular and glandular. Moreover, the histologic and cellular criteria as well as characteristic growth patterns provide reasonably good evidence for their different histogeneses. Inverted papillomas of the trabecular type develop by a proliferation of the basal cells of the transitional cell epithelium. Microcysts represent a pseudoglandular formation because the cells lining the cystic spaces are flattened urothelial cells and are incapable of producing mucus. The microcyst formation is most likely the result of cell necrosis within the core of the trabeculae and inverted papillomas of the trabecular type frequently disclose foci of squamous metaplasia. The glandular type of inverted papilloma develops from the so-called cystitis cystica and glandularis. Evidence in support of this concept is based particulary on their striking resemblance to those lesions. This closely resembles proliferative cystitis cystica and glandularis. Histogenetically inverted papillomas of the glandular type probably develop in a three-step process. The first step consists in the formation of von Brunn s cell nests, which may become increasingly cystic and progress into cystitis cystica. The second step is characterized by the transition of Brunn s cell nests or cystitis cystica into cystitis glandularis. Finally the third and most important step leads to a neoplastic transformation of cystitis cystica and glandularis, resulting in the formation of inverted glandular papillomas.

The biological behavior of inverted papillomas has been considered by most authors as benign because

of their histologic features and clinical course. However the current concepts of the benign nature of these tumors should be reevaluated, since malignant ones have been reported and those reports have suggested the possibility of malignant transformation. Altaffer et al. (1982) reported a frankly malignant inverted papilloma of the bladder and also association of transitional carcinoma in situ with an inverted papilloma. The case described by Lazarevic and Garret (1978) involved a transitional cell carcinoma arising from, or along with, an inverted papilloma. Uyama and Moriwaki (1981) reported a case that was partially a benign inverted papilloma and partially a medullary lesion of questionable malignancy and the case reported by Whitesel (1982) represented a papillary neoplasm arising from the overlying urothelium. We were able to observe the different pattern of inverted papilloma from benign, classical pattern of inverted papilloma in two cases. They exhibited increased cellularity, nuclear crowding and some dispolarity and composed of tissue that had markedly increased cell layers above the 6-8 cell thickness of normal urothelium. Those cellular abnormalities were interpreted as malignant transformation of the surface urothelial component of an inverted papilloma and malignant inverted papilloma or transitional cell carcinoma with a feature of inverted papilloma.

In this study, to gain a better understanding of the histopathological characteristics and biological potential of inverted papilloma of the bladder, we investigated the PCNA, NORs and c-erbB-2 oncoprotein that were established as indicators of the cellular proliferation activity. Among the various approaches to evaluating the prolifertive activity, recently, PCNA has been introduced as an antigenic marker of cellular proliferation. The studies with antibodies to this PCNA/Cyclin have shown the synthesis of this protein appears at the G1-S interphase and correlates directly with the proliferative state of normal and transformed cells. Recently, Dawson et al. (1990) shown that the PCNA/Cyclin staining was significantly greater in carcinoma compared to benign lesions after a comparative study of proliferation in 54 breast carcinomas and 15 benign breast lesions and PCNA/Cyclin staining were increased in moderately differentiated breast carcinoma compared to well-differentiated breast ones. In this study on seven inverted papillomas of the bladder, there was distinct difference of PCNA expression between benign and malignant inverted papilloma. The nuclei of the two malignant inverted papillomas exhibited a higher PCNA expression rate than the other five benign inverted papillomas of the bladder.

NORs, segments of DNA closely associated with nucleoli, contain coding genes for ribosomal RNA and contribute to the regulation of cellular protein synthesis. Nucleolar organizer regions are intimately associated with argyrophilic proteins, and a recent modification of a silver staining technique, long used by cytogeneticists, allows NORs to the visualized in conventional histologic sections, where they are called AgNORs. Malignant cells contain considerably more AgNORs than benign cells, and high grade lymphomas have significantly more AgNORs than low grade lymphomas (Crocker and Nar, 1987).

Dervan et al. (1989) concluded in their study of benign and malignant breast lesions using silver staining technique that AgNORs counts furnish significant kinetic information, and the difference in AgNORs counts between benign and malignant tumors may be of diagnostic value. The study on semiautomatic quantification of silver-stained nucleolar organizer regions by Ploton et al. (1992) demonstrated a significant difference in NORs numbers between two groups of prostatic caners with good and poor prognosis. Because nucleolar activity contributes significantly to cell proliferation, we studied the AgNORs to achieve kinetic difference of cells between benign and malignant inverted papillomas. Two malignant inverted paillomas demonstrated increased mean AgNORs counts, larger nucleoli and more variation in size of AgNORs than benign inverted papillomas of the bladder.

The human proto-oncogene c-erbB-2, also referred to as neu or HER-2, has close similarities with the c-erbB gene encoding the epidermal growth fator recepter(EGF-R). The c-erbB-2 gene product exhibits tyrosine kinase activity and appears to be a receptor for an certain growth factor. The c-erbB-2 gene product is expressed in urothelium, proximal and distal renal tubules, gastro-intestinal epithelium, and bronchial epithelium of the fetus, but has been scarcely detected in any normal adult tissues except for renal tubule using the same techniques (Mori et al., 1989). Recent work suggests that the c-erbB-2 oncogene plays an improtant role in carcinogenesis. The gene is amplified in approximately 30 percent of primary breast cancers and overexpression is associated with aggressive behavior (Slamon et al., 1987). Furthermore, amplified expression of HER2/neu in

transfected 3T3 cells induces malignant transformation (DiFore et al., 1987). Studies on expression of c-erbB-2 gene product in urinary bladder cancer showed that the c-erB-2 product may be applicable as a tumor showed that the c-erbB-2 product may be applicable as a tumor marker for evaluation of malignant potential, invasiveness, and probably metastatic potential of human bladder cancer, and merits as a potential prognosic indicator (Moriyama et al., 1991 ; Cooms et al., 1991). We therefore investigated the expression of the c-erbB-2 gene product in inverted papilloma specimens, and the correlations of their expression with their malignant potential. There was high degree of c-erbB-2 immunoreactivity only in malignant inverted papillomas of the bladder.

Whether these two inverted papillomas with malignant transformation are regarded as a malignant inverted papilloma or a transitional cell carcinoma with features of inverted papilloma would seem to be academic. In summary, the lesions microscopically resembling inverted papilloma deserve careful examination for cytological evidence of malignancy and careful followup with endoscopic examination may be indicated, and they cannot be considered routinely as benign proliferation. More detailed studies including prolonged followup are needed for assessment of the biological behavior of these tumors.

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