Review

Non-papillary Urothelial Lesions of the Urinary Bladder: Morphological Classification and Immunohistochemical Markers

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Abstract. *In the past quarter century, several nomenclature systems for non-papillary urothelial lesions of the urinary bladder have been proposed. The 1998 World Health Organization and International Society of Urological Pathology (WHO/ISUP) classification recommended a four-tier system for the subdivision of flat intraepithelial lesions of the bladder. This classification was adopted by the WHO in 1999 and revised in 2004. In 2002, a more detailed classification of flat urothelial lesions was based on the Ancona International Consultation. Each of these lesions was defined with strict morphological criteria to provide more accurate information to urologists in managing patients. However, immunohistochemical markers including proliferation markers, cytoskeletal proteins, growth factors and their related receptors, cell adhesion molecules and oncogene proteins are increasingly being used in order to differentiate histologically similar lesions.*

A. Molecular and Genetic Basis of Classification – Field Effect

The neoplastic transformation of the urothelium that lines the renal pelvis, the ureters, the urinary bladder and the urethra, is a complex process with myriad clinical and pathological manifestations, many of which may occur simultaneously or over time. It has been suggested that a "field" effect, possibly accounting for the multifocality of urothelial cancer, might result when the urothelium is bathed in growth factors or carcinogens present in urine. From the morphological point of view, two basic distinct but occasionally interrelated pathways are identified on the basis of the pattern of growth of the intraepithelial lesions (papillary and flat), the behaviour of these lesions being related to the degree of architectural and cytological alteration of the urothelium. "Superficial" lesions (80%) can be divided further into papillary cancer and the much less common "flat" lesions of high-grade carcinoma *in situ* (CIS). The remaining 20% of bladder carcinomas are non-papillary, invasive tumors at the time of initial presentation. Reflecting these two distinct clinical presentations of bladder cancer, molecular genetic studies have also demonstrated different molecular pathways during bladder cancer progression. P53 gene inactivation and loss of heterozygosity (LOH) of chromosome 17p appear to occur early in CIS and are frequent in invasive tumors, whereas selective deletions of chromosome 9q may be more commonly seen in papillary cancer. However chromosome 9 deletions cannot be regarded as indicators of papillary growth, because they are found frequently in both types of flat lesions of the urothelium: in those associated with papillary tumors and in those that are not. It has been also proposed that *p53* mutations (p53 overexpression) precede loss of chromosomes 9 and 9p21 in CIS as precursor for invasive bladder cancer, as opposed to noninvasive carcinomas where chromosome 9 (9p11-q12) losses are early and frequently combined with homozygous deletions of 9p21 (1-4).

B. Morphological Classification

A morphological continuum of progressive nuclear abnormalities that progress to CIS and invasive carcinoma is recognizable in the flat urothelium in animal models and patients with bladder neoplasia. Direct evidence that this sequence is the actual pathway of carcinogenesis has not been convincingly shown under prospective investigation and follow-up in humans. In the past quarter century, several nomenclature systems have been proposed (Table I) (3, 5-9).

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Key Words: Non-papillary lesions, urothelium, urinary bladder, immunohistochemistry, review.

Table I. *Nomenclature for flat intraepithelial lesions of urothelium*.*

Koss (5)	Nagy *et al*. (6)	Mostofi and Sesterhenn (7)	Koss *et al*. (8)	Murphy *et al*. (9)	Amin *et al*. (3)
Simple hyperplasia	Atypia, not otherwise classified	CIS, gI	IUN1	Dysplasia	Urothelial atypia, reactive
Atypical hyperplasia	Mild dysplasia	CIS, gII	IUN 2	CIS	Urothelial atypia, unknown significance
CIS	Moderate dysplasia	CIS, gIII	IUN 3		Urothelial dysplasia, low-grade
	Severe dysplasia				High-grade dysplasia/CIS
	CIS				

*There is no correlation among the columns. CIS: Carcinoma in situ; g: grade; IUN: Intra-urothelial neoplasia.

The 1998 World Health Organization and International Society of Urologic Pathology (WHO/ISUP) classification recommended a four-tier system for the subdivision of flat intraepithelial lesions of the bladder (Table II) (10). This classification was adopted by the WHO in 1999 and included later in "Pathology and Genetics of Tumors of the Urinary System and Male Genital Organs," one of the "Blue Books" of the new series of WHO Classification of Tumors (11, 12).

A more detailed revised classification of flat and endophytic lesions was published by Lopez-Beltran *et al*. in 2002 and referred to an update based on the Ancona International Consultation (Table III) (13). Each of these lesions was defined with strict morphological criteria to provide more accurate information to urologists in managing patients. The most recently revised classification of flat intraepithelial lesions is that of Montironi and Lopez-Beltran, referred to as 2004 WHO classification, which includes a classification of all bladder tumors (14).

The present review is based on the 1998 WHO/ISUP classification and combines the recent classifications.

1. Normal Mucosa

A minimally architecturally disordered flat urothelium with hyperchromatic nuclei due to vagaries of staining and fixation usually refer to normal urothelium and the frequently used term "mild dysplasia" should be avoided for these cases (4, 10).

2. "Benign" Epithelial Abnormalities

There are many urothelial "benign" epithelial abnormalities, the identification of which is not always straightforward because of the lack of precise morphological criteria (4, 13, 15).

Urothelial Atypia (Reactive Atypia, Reactive Changes)

Urothelial abnormalities whose architectural and cytological changes are of a lesser degree than in dysplasia have often been termed atypia. The use of the term urothelial atypia

Table II. *The 1998 WHO/ISUP classification (10).*

Normal
  Normal

Hyperplasia
  Flat hyperplasia
  (Papillary hyperplasia)

Flat lesions with atypia
  Reactive (inflammatory atypia)
  Atypia of unknown significance
  Dysplasia (low-grade intraurothelial neoplasia)
  Carcinoma *in situ* (high-grade intraurothelial neoplasia)

Table III. *Working classification of preneoplastic non-papillary intraepithelial lesions and conditions of the urothelium: the Ancona proposal, 2001 (13).*

Epithelial abnormalities
  Reactive urothelial atypia
  Flat urothelial hyperplasia

Presumed preneoplastic lesions and conditions
  Metaplasia of the urinary bladder
    Keratinizing squamous metaplasia
    Glandular metaplasia
  Malignancy-associated cellular changes

Preneoplastic lesion
  Dysplasia

Neoplastic non-invasive lesion
  Carcinoma *in situ*

without further specification to encompass reactive atypia is discouraged by Lopez-Beltran *et al*. (13).

Urothelial reactive (inflammatory) atypia. Urothelial reactive atypia (URA) occurs in acutely or chronically inflamed urothelium and is characterized by mild nuclear abnormalities (Figure 1 A). The cells are often larger than normal, with more

abundant cytoplasm. The nuclei are uniformly enlarged and vesicular with central prominent nucleoli. Mitotic figures may be frequent. Marked nuclear hyperchromasia, pleomorphism and irregularity of the chromatin pattern are lacking. Inflammatory cells in the lamina propria penetrating the urothelium are always present. In most cases, there is a history of instrumentation, stones, or previous therapy (10, 13).

Urothelial atypia of unknown (uncertain) significance. In some cases it is difficult to differentiate between reactive and neoplastic atypia. The term "urothelial atypia of unknown significance (UAUS)" was introduced by the WHO/ISUP consensus group to describe lesions in which the pathologist is uncertain whether the changes are reactive or preneoplastic, but is considered the most controversial and least understood category of intraurothelial lesions (Figure 1 B). UAUS shows nuclear changes similar to those seen in URA. The degree of nuclear pleomorphism and/or hyperchromasia is greater than in the latter and out of proportion to the extent of the inflammation, such that dysplasia cannot be ruled out with certainty. UAUS is often seen in patients with a previous diagnosis of urothelial neoplasms. Progression to urothelial carcinoma has not been documented and there is no evidence supporting a premalignant nature in such lesions. Patients need to be followed more closely and re-evaluated once the inflammation subsides (10, 13). Cheng *et al*. hold that the clinical outcomes of patients with UAUS are not different from those of patients with URA and recommend that the diagnostic category of UAUS should be eliminated in the next future classification. They believe that it might be practical to combine UAUS with URA in order to convey meaningful information to the patients and urologists (16).

Flat Urothelial Hyperplasia

Flat urothelial hyperplasia (FUH) consists of a markedly thickened mucosa without cytological atypia. Usually ten or more cell layers characterize the lesion, but at least more than seven layers are necessary for this diagnosis. Slight nuclear enlargement may be focally present. Morphological evidence of maturation from base to surface is generally evident. This lesion may be associated with a wide variety of inflammatory disorders and lithiasis and may be seen adjacent to dysplasia, CIS and low-grade papillary urothelial neoplasms (10, 13). In a high percentage of cases (71%), FUH has the same chromosome 9 deletions as low-grade papillary carcinoma. In contrast, 17p13 deletion is found in a low percentage (8%) of FUH and low-grade urothelial carcinomas. FUH could be the source of papillary neoplasia, but as it can display many genetic alterations commonly found in bladder cancer, it may also be an early neoplastic lesion in the multistep development of invasive urothelial carcinoma. However, when seen by itself there is no data suggesting that it has any premalignant potential (13, 17, 18).

3. Presumed Preneoplastic Lesions and Conditions

Preneoplastic Conditions and Lesions other than Urothelial (Metaplasias)

Squamous and glandular metaplasia arise in the urothelium as a response to chronic irritation and may co-exist. Although they are benign lesions, they can be considered preneoplastic conditions, since cancer has been observed either in the follow-up of some patients or in association with these lesions. They become preneoplastic lesions when specific cells changes occur (13).

Keratinizing squamous metaplasia. Keratinizing squamous metaplasia (KSM) can develop into squamous cell carcinoma (SCC), with intraepithelial dysplastic changes and carcinoma *in situ* as intermediate steps. Usually these lesions are flat and whitish in color cystoscopically. The exophytic pattern of KSM (squamous cell papilloma) may be related to the development of verrucous carcinoma. Schistosomiasis of the urinary bladder is frequently associated with KSM and SCC (13). Nonkeratinizing squamous epithelium resembles vaginal epithelium due to prominent glucogenation, is normally found in the trigone in 75-86% of women and should not be termed metaplasia (13).

Glandular metaplasia. Glandular metaplasia (GM) is characterized by the presence of epithelial cells of colonic type with a goblet cell appearance (or rarely that of Paneth, argentaffin, or argyrophil cells) within the surface epithelium (flat pattern) and/or in association with cystitis cystica (endophytic pattern). GM may present as an exophytic lesion with dysplasia, usually called villous adenoma. The development of adenocarcinoma from these lesions has been documented in the long-term follow-up of very few patients. Still, such lesions have been considered preneoplastic conditions. Intestinal metaplasia refers to mucin-secreting metaplasia that shows a striking resemblance to intestinal mucosa, including a crypt-like architecture. It may be focal or diffuse, the second type being associated with an increased risk of development of adenocarcinoma. Nuclear pleomorphism and prominent nucleoli are considered indicative of malignant change (glandular dysplasia and adenocarcinoma *in situ*) and may reflect intermediate steps between intestinal metaplasia and invasive adenocarcinoma (13).

Malignancy-associated Changes

The concept of malignancy-associated change (MAC) was introduced by Lopez-Beltran *et al*. to encompass those epithelial abnormalities that are present in urinary bladders harboring preneoplastic and neoplastic lesions, and that are not detectable by routine light microscopic examination (13). With the use of

image analysis, subtle morphological nuclear abnormalities that are not readily seen by the human eyes have been demonstrated. Recent studies showed that 50% of the histologically normal urothelium adjacent to superficial urothelial carcinoma has genetic anomalies on chromosome 9, similar to the anomalies found in the coexisting carcinoma (18-22).

MAC and FUH are considered to be the earliest detectable steps in the development of bladder cancer (4, 13).

4. Preneoplastic Lesions

Urothelial Dysplasia

Dysplastic urothelium has appreciable cytological and architectural changes felt to be preneoplastic, yet falling short of the diagnostic threshold for urothelial CIS (Figure 1 C). Low-grade intraurothelial neoplasia, atypical hyperplasia, intraepithelial neoplasia grades I to II, intraurothelial neoplasia I to II, dysplasia grades I to II, and mild-moderate dysplasia have been previously used for the term urothelial dysplasia (UD). Studies based on morphological observations in biopsies from patients with urothelial neoplasia as well as observations in the animal model system of Wistar rats show that the atypia in the flat intraepithelial lesions of the urinary bladder "progresses" qualitatively and quantitatively, stressing the importance of cytological features rather than of the histological pattern. This means using the term UD without a qualifier is preferable to describing the morphological spectrum of dysplastic lesions of the urothelium (3, 10, 13).

UD consists of cohesive cells, characterized by mild nuclear/nucleolar changes that focally include irregular nuclear crowding, slight hyperchromasia and anisonucleosis. Umbrella cells are usually present. There may be an increased number of cell layers, nucleoli may be prominent and mitotic figures when present are generally basally located. Most cellular abnormalities in UD are restricted to the basal and intermediate layers. UD shares certain morphological and genetical features with CIS. Nuclear and architectural features are considered most useful in distinguishing URA and UD (10, 13, 23, 24).

Dysplasia may be primary or secondary. *Primary UD*. This occurs in the absence of other urothelial tumors. Patients with primary UD are predominantly middle-aged men with irritative bladder symptoms with or without hematuria. Cystoscopically the urothelium may appear normal, raised and irregular or mildly erythematous. Only a low percentage of cases (13%) will progress to CIS (13). *Secondary UD*. This occurs in patients with bladder neoplasia. Its incidence varies from 22% to 86% and approaches 100% in patients with invasive carcinoma. UD in patients with noninvasive UC appears to be a marker for recurrence and progression (13).

5. Neoplastic Noninvasive Lesions

Urothelial Carcinoma *In Situ*

CIS refers to a flat noninvasive neoplastic change of the urothelium with substantial architectural and cytological abnormalities (Figure 1 D). The terms high-grade intraurothelial neoplasia, severe dysplasia, grade III CIS, high-grade dysplasia and grade III intraurothelial neoplasia have been previously used for CIS. The morphological diagnosis of CIS requires the presence of severe cytological atypia (nuclear anaplasia). The lesion may be only one layer thick, of normal thickness, or hyperplastic. Full-thickness change is not essential, although it is usually present. Prominent disorganization of cells is characteristic, with loss of polarity and cohesiveness. Superficial (umbrella) cells may be present except in areas of full-thickness abnormality. CIS is characterized by the presence of large and pleomorphic cells with moderate to abundant cytoplasm and large, irregular, hyperchromatic nuclei. Sometimes the cells are small with a high nucleus to cytoplasm ratio. The chromatin tends to be coarse and clumped. Nucleoli are usually large and prominent in at least some of the cells, and may be multiple. Mitotic figures that may be atypical are often obvious, mainly in the mid to upper urothelium. Morphometrically, the cells in CIS display an increase in the nuclear area, nuclear perimeter, and maximum nuclear diameter. Tissue edema, vascular ectasia, and proliferation of small capillaries are frequent in the lamina propria (10, 13, 23-25).

A number of histological patterns have been described: small cell CIS, large cell CIS (with pleomorphism and without pleomorphism), "denuding cystitis" and "clinging" CIS, undermining (lepidic) growth, pagetoid CIS, CIS involving Von Brunn nests and CIS involving prostatic urethra or ureter (10, 13, 25). The small cell CIS pattern is unrelated to neuroendocrine differentiation. Striking cellular discohesion in some cases results in few ("clinging") or no recognizable epithelial cells on the surface, a condition referred to as denuding cystitis of CIS. In denuded areas, CIS may only be present in Von Brunn nests, a useful clue in the diagnosis of residual CIS (26). Rarely, CIS exhibits pagetoid growth, characterized by an intraepithelial proliferation of large cells arranged singly or in clusters and randomly distributed. The pagetoid variant of CIS deserves recognition and attention, chiefly because it may be overlooked or misdiagnosed as urothelial dysplasia, then causing unsuspected tumor recurrence after surgery. Given that primary extramammary Paget disease of the external genitalia and of the anal canal may extend to the bladder and, conversely, some bladder cases of pagetoid CIS may extend to the urethra, ureter, and beyond to the external genitalia, the differential diagnosis between these two entities represent an important therapeutic consideration. The two lesions can be differentiated immunohistochemically, as the pagetoid CIS is CK7+/CK20+ and the Paget disease is known

to be CK7+/CK20– (13, 27). The clinical significance, if any, of the CIS patterns remains to be determined, and in the pathology report, all of these patterns should be diagnosed as urothelial CIS with no specific mention of the morphological pattern. Increased awareness of the morphological heterogeneity of CIS will facilitate its separation from its histological mimics (15).

CIS may be primary or secondary. *Primary CIS*. Primary CIS occurs in the absence of other urothelial tumors, almost exclusively in men over 50 years old and accounts for fewer than 10% of cases of CIS and 1% of bladder tumors. Dysuria, nocturia, and sterile pyuria are the main symptoms. In approximately 25% of the patients, primary CIS is asymptomatic. Cystoscopically, it appears as erythematous, velvety, or granular patches, although it may be visually undetectable. The trigone, the floor and the neck of the bladder are the main sites of involvement, though the whole mucosa can be affected and the lesion is multifocal in over 50% of the cases. The prostatic urethra and the ureters are involved in 67% and 57% of cases respectively (13).

Secondary CIS. Secondary CIS is more common than primary CIS, accounting for 90% of cases. It is associated with a prior, or synchronous noninvasive, or invasive urothelial carcinoma. The frequency of CIS increases with the grade and stage of the associated neoplasm. As many as 24% of random biopsies from patients with Ta and T1 carcinoma show epithelial abnormalities that include dysplasia and CIS (13, 16).

In most cases, CIS is multifocal and is very likely to progress to invasive carcinoma if left untreated. The mean interval from diagnosis to progression is 5 years. Primary CIS has a lower risk of progression (28% *vs.* 59%) and death rate (7% *vs.* 45%) than secondary CIS. Factors predictive of a high risk of progression include multifocality, coexistent bladder neoplasm, prostatic urethral involvement and recurrence after treatment (13, 25).

CIS with microinvasion. Microinvasive carcinoma of the urinary bladder is defined as invasion into the lamina propria to a depth ranging 2-5 mm from the basement membrane. Microinvasion often appears as cords of direct extension, single cells or clusters of cells. The microinvasive component into the lamina propria should constitute no more than 20 invading cells, measured from the stroma-epithelial interface. The use of immunohistochemistry with antibodies against cytokeratins may help in the identification of invading cells associated with chronic inflammation (10, 13).

C. Mimics of Urothelial Flat Neoplasia

Overdiagnosis of UD and CIS can be made in cases of inflammatory conditions of the urinary bladder, often related to the effects of treatment. Marked atypia of the urothelium may be induced after the treatment of CIS with intravesical chemotherapy (*e.g.* mitomycin C, thio-TEPA) and treatment with BCG. The atypia associated with intravesical chemotherapy is mainly observed in the superficial layer of the urothelium. It should be noted that after BCG intravesical infusions, residual CIS may remain only in Von Brunn nests. Systemic chemotherapy for non-urological malignancies may cause severe regenerative atypia of the urothelium. A widespread denudation of the urothelium, with a variably retained single cell layer of very atypical large cells is often observed, giving the impression of the clinging pattern of CIS. The atypical urothelium is commonly accompanied by an inflamed, edematous, sometimes hemorrhagic lamina propria that may have atypical stromal cells (giant cell cystitis). The urothelial atypia caused after pelvic radiotherapy has a degenerative appearance with smudged, hyperchromatic, multilobulated nuclei, nuclear and cytoplasmic vacuolization and a ballooning of the cytoplasm producing a low nuclear to cytoplasmic ratio. Knowledge of the prior treatment is necessary in order to make the correct diagnosis. If the distinction between treatment-induced atypia and dysplasia/CIS is uncertain, a conservative approach with repeat cystoscopy and biopsy, preferably after inflammation has subsided, is indicated. Polyoma virus infection can mimic flat intraepithelial lesions in immunodepressed patients (13).

Additional epithelial abnormalities (endophytic). Von Brunn nests and cystitis cystica, even though listed under epithelial abnormalities, are benign endophytic conditions that usually do not represent a problem from the diagnostic point of view. Their epithelial lining might show features of reactive URA, UD and CIS identical to those of the flat urothelium (13).

D. Immunohistochemical Diagnostic and Prognostic Markers

The interobserver reproducibility using the current WHO/ISUP classification for flat lesions of the urinary bladder should be investigated. CIS is now a recognized precursor of invasive bladder cancer and its presence in a patient with a coexisting noninvasive papillary urothelial carcinoma has a significant negative influence on the clinical outcome. The diagnosis of CIS in bladder biopsy specimens is thus crucial because it not only alters prognosis but often alters the subsequent therapy as well. Furthermore, it has recently been demonstrated that isolated UD is also associated with a risk of both CIS and invasive bladder cancer. Diagnostic problems in the distinction between URA, UAUS, and UD, as well as the distinction of UD from CIS, are unresolved. Additionally, the morphology of urothelial CIS may overlap with that of URA, resulting in diagnostic difficulty when interpreting bladder biopsies of patients on surveillance for urothelial neoplasia. Furthermore, follow-up information on patients diagnosed with these atypical urothelial proliferations is
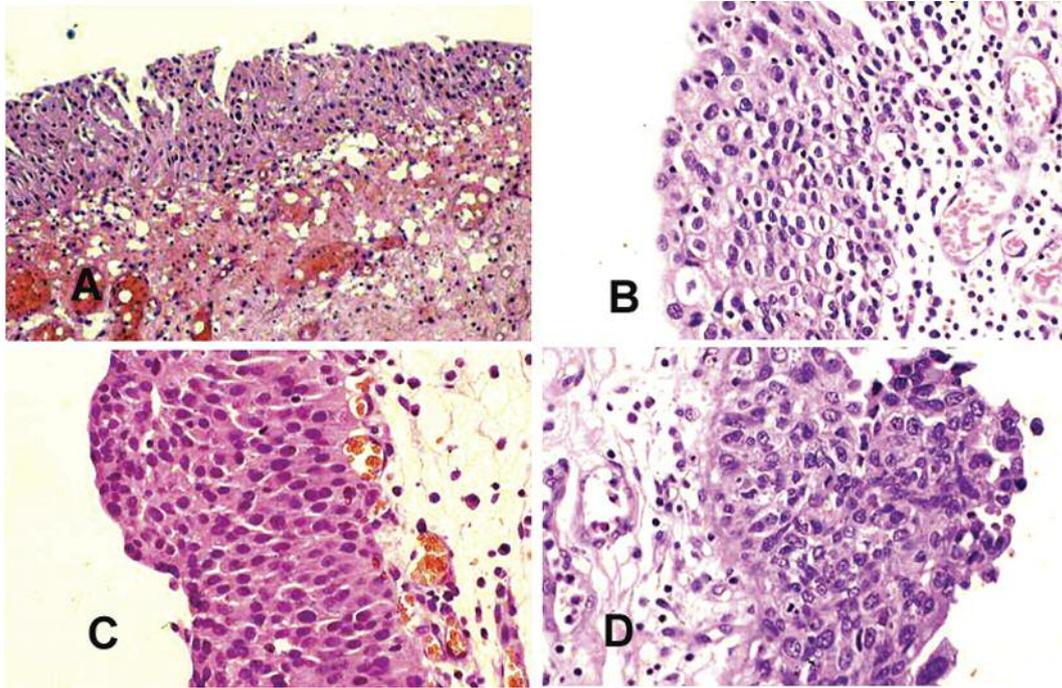
Figure 1. *A, Urothelial reactive atypia (H-E ×100); B, Urothelial atypia of unknown significance (H-E ×200); C, Urothelial dysplasia (H-E ×200); D, Urothelial carcinoma in situ (H-E ×200).*
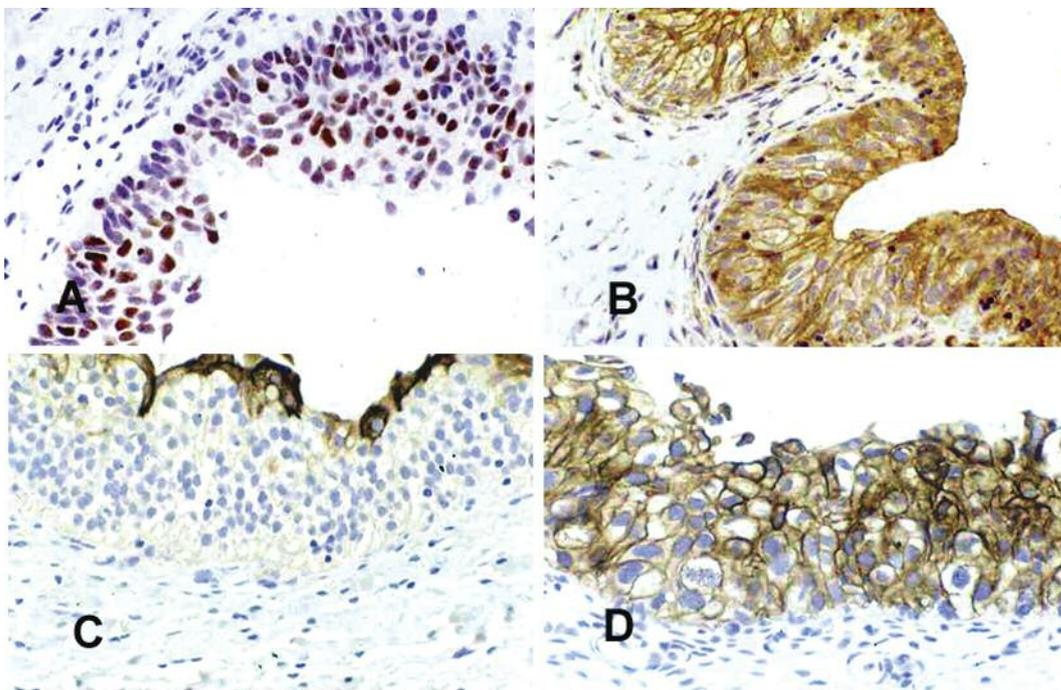

Figure 2. *A, Nuclear p53 immunohistochemical overexpression in all cell layers in CIS; B, Membranous, full-thickness c-erbB-2 immunoexpression in CIS; C, Membranous CK20 immunoexpression in the superficial cells of normal urothelium; D, Membranous, full-thickness CK20 immunoexpression in CIS (All magnifications ×200).*

limited. Further refinement of diagnostic criteria and development of quantitative measures (such as immunohistochemical, morphometric and molecular markers) should be considered in an effort to reach complete consensus among pathologists (16). Multiple markers of bladder neoplasia have been investigated with respect to tumor diagnosis, recurrence, progression to invasive disease and response to therapy. These include proliferation markers, cytoskeletal proteins, growth factors and their related receptors, cell adhesion molecules and oncogene proteins. It has been suggested in some studies that abnormal immunohistochemical expression of several of these markers may have a utility in non-papillary bladder lesions detected in the presence or in the absence of cancer. Furthermore, some studies have demonstrated that according to the concept of the field change, phenotypic changes suggestive of malignancy occur in morphologically benign urothelium in patients with a history of bladder cancer (23, 28).

1. Diagnostic Markers

Although conventional histological examination has been the usual method of defining flat lesions of the urothelium, immunohistochemical markers are increasingly being used to differentiate histologically similar lesions.

Proliferation Markers

Ki-67/MIB1. In the normal urothelium, in FUH and in URA, nuclear Ki-67/MIB1 is more often expressed in the basal and occasionally in the lower part of the middle layers. The MIB1 pattern of UD corresponds to that of URA. In CIS, MIB1 labeling is seen in all urothelial layers (11, 29-33).

Oncogenes and Oncosuppressor Genes

Bcl2. Bcl2 is expressed predominantly in the basal cells of the urothelium (28). In normal urothelium and URA, Bcl2 is restricted to the basal cell compartment, while in UD and CIS its expression is detectable also in the upper regions. Significant reciprocal correlation is found between Bcl2 and p53 expression in UD (34).

p53. In the normal urothelium, p53 nuclear immunoreactivity varies from none to focal and very weak. p53 staining in URA varies from absent staining to patchy and weak nuclear reactivity, predominantly in the basal cell layer. UD and CIS show diffusely distributed overexpression of p53 throughout all cell layers in the majority (>50%) of neoplastic cells in most cases (Figure 2 A). Overexpression of p53 protein shows an increasing trend toward the progression of bladder tumorigenesis (15, 29, 33, 34).

Class I Tyrosine Growth Factor Receptors

Epithelial growth factor receptor (EGFR). EGFR staining is predominantly membranous, although a low level of cytoplasmic staining is sometimes present. It is mostly restricted to the basal cells of normal epithelium (30). However a variable fraction of EGFR-reactive cells is also referred to, ranging from a positivity limited to the basal cell layer to a positive diffuse staining spanning the entire thickness of the urothelium. There is no clear association between basal or diffuse EGFR staining and the degree of histological atypia (29, 35).

Erb2 (c-erbB-2, HER-2/neu), ErbB3 and ErbB4. Benign urothelium shows a membranous expression of c-erbB-2 by the superficial cell layer and occasionally by some intermediate cells. The immunostaining is rather strong on the basolateral side of the cell membrane. A diffuse membranous, full-thickness staining pattern is most exclusively observed in UD and CIS (Figure 2 B). ErbB3 tends to be distributed on the superficial cells, although some is noted throughout the urothelium and ErbB4 is present predominantly in the superficial layer (29, 30).

Intermediate-filament Polypeptides/Cytokeratins

Cytokeratin 20. The expression of CK20 is cytoplasmic and restricted to the differentiated superficial 'umbrella' cells and occasional intermediate cells in the normal urothelium (Figure 2 C). Urothelium with URA also shows CK20 staining in only the umbrella cell layer. In cases of UD and CIS, complete loss of restriction is seen, at least focally, with positive expression in all layers of the urothelium (Figure 2 D). This means that in dysplastic urothelium, the expression of CK20 becomes dysregulated rather than lost. These results confirm the potential of CK20 as a 'positive' marker of abnormal urothelial cell phenotype (15, 31, 33, 36).

34βE12 (anti-CK1, CK5, CK10, CK14). In H-E stained slides, normal urothelium, FUH and URA show a prominent basal cell layer, located on a clearly distinct basal membrane. In UD, the basal cell layer is less prominent or even fragmented and is completely missing in CIS. 34βE12 Immunohistochemistry parallels these findings, whereas 34βE12 immunohistochemistry in hyperplastic and reactive atypical urothelium shows continuous basal cell layer staining. The staining pattern in UD is less prominent, in part fragmented. It is always confined to basal and the lower part of the middle layers in the urothelium. In CIS, a diffuse 34βE12 staining is seen in all urothelial layers. The staining pattern of MIB1 parallels that of 34βE12 (11, 23).

Cell Adhesion Molecules

CD44. In the normal urothelium, CD44 shows consistent but patchy membranous staining of only the basal cell layer. Its staining pattern varies from membranous overexpression in the full thickness of reactive urothelium in some cases to patchy positivity in the basal and intermediate cells (similar to normal urothelium) in other cases. The cases of URA with the most marked cytological changes show the strongest full-thickness CD44 immunoreactivity. There is a lack of CD44 reactivity in the neoplastic cells of CIS, although an underlying residual basal cell layer, which shows CD44 membranous reactivity, is present in some cases (15).

2. Prognostic Markers

Biomarkers of prognosis have not yet been systematically evaluated in premalignant urothelial disease. Immunohisto-chemical assessment for *p53* shows that this gene is frequently overexpressed (61%) in CIS. Patients whose tumors overexpress p53 are at a significantly increased risk of tumor progression.

There is relatively little information on how treatment modifies the information gained from biomarker analysis. Immunohistochemical expression of p53 does not predict response to BCG treatment. However, immunohistochemical detection of altered p53 expression in bladder cancer after BCG treatment is significantly associated with a high risk of disease progression (28, 37).

Conclusion

The differential diagnosis of flat intraepithelial lesions of the urothelium may cause difficulties. Evaluation of the clinical history, the cystoscopical findings and the morphological features of the lesion usually leads to the correct diagnosis. The use of several immunohistochemical markers may help in distinguishing morphologically similar lesions.

References

1 Hartmann A, Schlake G, Zaak D, Hungerhuber E, Hofstetter A, Hofstaedter F and Knuchel R: Occurence of chromosome 9 and p53 alterations in multifocal dysplasia and carcinoma *in situ* of human urinary bladder. Cancer Res *62*: 809-818, 2002.

2 Hopman AHN, Kamps MAF, Speel EJM, Schapers RFM, Sauter G and Ramaekers FCS: Identification of chromosome 9 alterations and p53 accumulation in isolated carcinoma *in situ* of the urinary bladder *versus* carcinoma *in situ* associated with carcinoma. Am J Pathol *161*: 1119-1125, 2002.

3 Amin MB and Young RH: Intraepithelial lesions of the urinary bladder with a discussion of the histogenesis of urothelial neoplasia. Sem Diagn Pathol *14*: 84-97, 1997.

4 Montironi R, Lopez-Beltran A, Mazzucchelli R and Bostwick DG: Classification and grading of the non-invasive urothelial neoplasms: recent advances and controversies. J Clin Pathol *56*: 91-95, 2003.

5 Koss LG: Precancerous lesions of the urothelium. *In*: Atlas of Tumor Pathology. Tumors of the Urinary Bladder. Firminger HI (ed.). 2nd series. Fascicle 11. Washington, D.C. Armed Forces Institute of Pathology, pp. 62-70, 1975.

6 Nagy GK, Frable WJ and Murphy WM: Classification of premalignant urothelial abnormalities. A Delphi study of the National Bladder Cancer Collaborative Group A. Pathol Annu *17*: 219-233, 1982.

7 Mostofi FK and Sesterhenn IA: Pathology of epithelial tumors and carcinoma *in situ* of bladder. Prog Clin Biol Res *162*: 55-74, 1984.

8 Koss LG: Bladder cancer from a perspective of 40 years. J Cell Biochem Suppl *161*: 23-29, 1992.

9 Murphy WM, Beckwith JB and Farrow GM: Tumors of the urinary bladder. *In*: Atlas of Tumor Pathology. Tumors of the Kidney, Bladder and Related Urinary Structures. Rosai J and Sobin LH (eds.). 3rd series. Fascicle 11. Washington, D. C. Armed Forces Institute of Pathology, pp. 193-297, 1994.

10 Epstein JI, Amin MB, Reuter VR, Mostofi FK and the Bladder Consensus Conference Committee: The World Health Organization/International Society of Urological Pathology Consensus Classification of Urothelial (Transitional Cell) Neoplasms of the Urinary Bladder. Am J Surg Path *22*: 1435-1448, 1998.

11 Mostofi FK, Davis CJ and Sesterhenn IA (eds.). Histological Typing of Urinary Bladder Tumors. World Health Organization International Histological Classification of Tumors, 2nd ed. Berlin, Springer-Verlag, 1999.

12 Sauter G, Algaba F, Amin MB, Busch C, Cheville J, Gasser T, Grignon D, Hofstaedter F, Lope-Beltran A and Epstein JI: Non-invasive urothelial tumours: WHO classification of noninvasive papillary urothelial tumors. *In*: WHO Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Elbe JN, Sauter G, Epstein JI and Sesterhenn I (eds.). Lyon, IARCC Press, pp. 110, 2004.

13 Lopez-Beltran A, Cheng L, Andersson L, Brausi M, de Matteis A, Montironi R, Sesterhenn I, van der Kwast T and Marzerolles C: Preneoplastic non-papillary lesions and conditions of the urinary bladder: an update based on the Ancona International Consultation. Virchows Arch *440*: 3-11, 2002.

14 Montironi R and Lopez-Beltran A: The 2004 WHO Classification of bladder tumors: a summary and commentary. Int J Surg Pathol *13*: 143-153, 2004.

15 McKenney JK, Desai S, Cohen C and Amin MB: Discriminatory immunohistochemical staining of urothelial carcinoma *in situ* and non-neoplastic urothelium. An analysis of cytokeratin 20, p53, and CD44 antigens. Am J Surg Pathol *25*: 1074-1078, 2001.

16 Cheng L, Cheville JC, Neumann RM and Bostwick DG: Flat intraepithelial lesions of the urinary bladder. Cancer *88*: 625-631, 2000.

17 Obermann EC, Junker K, Stoehr R, Dietmaier W, Zaak D, Schubert J, Hofstaedter F, Knuechel R and Hartmann A: Frequent genetic alterations in flat urothelial hyperplasias and concomitant papillary bladder cancer as detected by CGH, LOH, and FISH analyses. J Pathol *199*: 50-57, 2003.

18 Baud E, Catilina P, Boitux JP and Bignon YJ: Human bladder cancers and normal bladder mucosa present the same hot spot of heterozygous chromosome-9 deletion. In J Cancer *77*: 821-824, 1998.

19 Baithun SI, Naase M, Blanes A and Diaz-Cano SJ: Molecular and kinetic features of transitional cell carcinomas of the bladder: biological and clinical implications. Virchows Arch *438*: 289-297, 2001.

20 Lee E, Schwaibold H, Fradet Y, Huland E and Huland H: Tumor-associated antigens in normal mucosa of patients with superficial transitional cell carcinoma of the bladder. J Urol *157*: 1070-1073, 1997.

21 Mazzucchelli R, Barbisan F, Stramazzotti D, Montironi R, Lopez-Beltran A and Scarpelli M: Chromosomal abnormalities in macroscopically normal urothelium in patients with bladder pT1 and pT2a urothelial carcinoma: a fluorescence *in situ* hybridization study and correlation with histologic features. Anal Quant Cytol Histol *27*: 143-151, 2005.

22 Montironi R, Filho AL, Santinelli A, Mazzuchelli R, Pomante R, Colanzi P and Scarpelli M: Nuclear changes in the normal-looking columnar epithelium adjacent to and distant from prostatic intraepithelial neoplasia and prostate cancer: morphometric analysis in whole-mount sections. Virchows Arch *437*: 625-634, 2000.

23 Helpap B and Koellermann J: Assessment of basal cell status and proliferative patterns in flat and papillary urothelial lesions: a contribution to the new WHO classification of urothelial tumors of the urinary bladder. Hum Pathol *31*: 745-750, 2000.

24 Murphy WM, Busch C and Algaba F: Intraepithelial lesions of urinary bladder: morphologic considerations. Scand J Urol Nephrol Suppl *205*: 67-81, 2000.

25 McKenney JK, Gomez JA, Desai S, Lee MW and Amin MB: Morphologic expressions of urothelial carcinoma *in situ*. A detailed evaluation of its histologic patterns with emphasis on carcinoma *in situ* with microinvasion. Am J Surg Pathol *25*: 356-362, 2001.

26 Levi AW, Potter SR, Schoenberg MP and Epstein JI: Clinical significance of denuded urothelium in bladder biopsy. J Urol *166*: 457-460, 2001.

27 Lopez-Beltran A, Luque RJ, Moreno A, Bollito E, Carmona E and Montironi R: The pagetoid variant of bladder urothelial carcinoma *in situ*. A clinicopathological study of 11 cases. Virchows Arch *441*: 148-153, 2002.

28 Grossman HB, Schmitz-Draeger B, Fradet Y and Tribukait B: Use of markers in defining urothelial premalignant and malignant conditions. Scand J Urol Nephrol Suppl *205*: 94-104, 2000.

29 Wagner U, Sauter G, Moch H, Novotna H, Epper R, Mihatsch J and Waldman FM: Patterns of p53, cerbB-2, and EGF-r expression in premalignant lesions of the urinary bladder. Hum Pathol *26*: 970-978, 1995.

30 Chow N, Liu H, Yang H, Chan S and Su I: Expression patterns of erbB receptor family in normal urothelium and transitional cell carcinoma. An immunohistochemical study. Virchows Arch *430*: 461-466, 1997.

31 Kunju LP, Lee CT, Montie J and Shah RB: Utility of cytokeratin 20 and Ki-67 as markers of urothelial dysplasia. Pathol Int *55*: 248-254, 2005.

32 Limas C: Proliferative state of the urothelium with benign and atypical changes. Correlation with transferring and epidermal growth factor receptors and blood group antigens. J Pathol *171*: 39-47, 1993.

33 Mallofre C, Castillo M, Morente V and Sole M: Immunohisto-chemical expression of CK20, p53, and Ki-67 as objective markers of urothelial dysplasia. Mod Pathol *16*: 187-191, 2003.

34 Li B, Kanamaru H, Noriki S, Yamaguchi T, Fukuda M and Okada K: Reciprocal expression of bcl2 and p53 oncoproteins in urothelial dysplasia and carcinoma of the urinary bladder. Urol Res *26*: 235-241, 1998.

35 Rotterud R, Nesland JM, Berner A and Fossa SD: Expression of the epidermal growth factor receptor family in normal and malignant urothelium. BJU Int *95*: 1344-1350, 2005.

36 Harnden P, Eardley I, Joyce AD and Southgate J: Cytokeratin 20 as an objective marker of urothelial dysplasia. Br J Urol *78*: 870-875, 1996.

37 Shariat SF, Kim J, Raptidis G, Ayala GE and Lerner SP: Association of p53 and p21 expression with clinical outcome in patients with carcinoma *in situ* of the urinary bladder. Urology *61*: 1140-1145, 2003.