The 2004 World Health Organization classification system for urothelial neoplasia identifies urothelial dysplasia (low-grade intraurothelial neoplasia) as a premalignant lesion of the urothelium. Although diagnostic criteria of urothelial dysplasia have been improved in recent years, there is a frequent lack of interobserver reproducibility. Follow-up studies suggest that dysplasia is a marker for urothelial instability and disease progression in up to 19% of patients, thus supporting an active clinical follow-up in these patients. The main differential diagnosis of urothelial dysplasia includes flat urothelial lesions with atypia, mainly flat (simple) urothelial hyperplasia, reactive urothelial atypia, urothelial atypia of unknown significance, and urothelial carcinoma in situ (high-grade intraurothelial neoplasia). In most cases, morphologic features alone suffice for diagnosis. Some cases may require a panel of immunohistochemical antibodies consisting of cytokeratin 20, p53 and CD44 for diagnosis. We present pathologic features and clinical significance of urothelial dysplasia with emphasis on differential diagnosis from common flat urothelial lesions with atypia. (Anal Quant Cytopathol Histopathol 2013;35:121–129)

Keywords: carcinoma in situ, diagnosis, intraepithelial neoplasms, pathology, urothelial dysplasia, urothelium.

Bladder cancer is a significant public health problem worldwide. It is the fourth most common cancer in men, accounting for 7% of all cancers. The typical cost per bladder cancer patient from diagnosis to death was estimated to be the highest among all cancers. Such high costs are due, in part,
to the high propensity for recurrence and progression that is characteristic of bladder cancer.\textsuperscript{2-13} Early identification of lesions may reduce costs and eventually lead to decreased bladder cancer morbidity and mortality.\textsuperscript{11}

As our understanding of bladder cancer has grown, several classifications of urothelial neoplasia have been proposed.\textsuperscript{2-19} In 1998 the International Society of Urological Pathology (ISUP), in association with the World Health Organization (WHO), developed a revised system for classifying flat and noninvasive papillary lesions.\textsuperscript{3} The 1998 ISUP/WHO system was adopted in 2004 for the WHO publication \textit{Pathology and Genetics of Tumors of the Urinary System and Male Genital Organs}.\textsuperscript{3,10} In addition to urothelial dysplasia (low-grade intraurothelial neoplasia), the 2004 WHO classification covers the nomenclature and histological findings of the following flat preneoplastic urothelial lesions: (1) flat urothelial hyperplasia, (2) reactive urothelial atypia, (3) urothelial atypia of unknown significance, and (4) carcinoma in situ (CIS; high-grade intraurothelial neoplasia) (Table I). The purpose of this article is to review the clinical significance and pathologic features of urothelial dysplasia with emphasis on differential diagnosis from other flat urothelial lesions with atypia.

**Urothelial Dysplasia**

Urothelial dysplasia, also known as low-grade intraurothelial neoplasia, is defined as abnormal urothelium with distinctive cytologic and architectural changes that do not meet all the criteria for the unequivocal diagnosis of urothelial CIS.\textsuperscript{2,3} In these cases the urothelium demonstrates significant cyto-

 logic atypia that cannot be attributed to inflammation or a reparative process (Figure 1).\textsuperscript{2,3}

**Pathologic Features**

The thickness of the urothelium may be normal, increased, or decreased, exemplifying the many faces in which urothelial dysplasia can appear in the daily practice of uropathology (Table II).\textsuperscript{2-22} Umbrella cells most commonly present. Cytologic abnormalities, including cellular crowding, loss of orderly maturation, and loss of polarity, are restricted to the basal and intermediate cell layers.\textsuperscript{2,3,20-22} Individual dysplastic cells show nuclear enlargement and occasional conspicuous nucleoli with irregular notched contours and coarse chromatin.\textsuperscript{2,3} Multiple nucleoli and nuclear overlapping may be seen. Mitoses, when present, are generally basally located. In practical terms, scoring the percentage of Ki-67 positive nuclei is superior to mitotic count and the mitosis marker phosphohistone H3 in terms of differentiating flat lesions of the bladder.\textsuperscript{23} The transition from normal to abnormal urothelium is subtle, and normal urothelial cells can be seen dispersed amongst the dysplastic cells. Pathologic diagnosis of urothelial dysplasia is based primarily in nuclear and architectural features.\textsuperscript{2,3,20}

The histologic criteria for distinguishing severe dysplasia from CIS are unreliable in most cases, with frequent lack of reproducibility in most studies (Table II).\textsuperscript{2,3,17} It is also difficult to distinguish mild dysplasia from moderate dysplasia. Recognizing these limitations, it is recommended that severe dysplasia and CIS be combined into a single category.\textsuperscript{2,3} It is also recommended that dysplasia not be

<table>
<thead>
<tr>
<th>2004 WHO classification</th>
<th>Clinical significance</th>
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<tbody>
<tr>
<td><strong>Reactive atypia/Atypia of unknown significance</strong></td>
<td>None developed dysplasia, carcinoma in situ or urothelial carcinoma</td>
</tr>
<tr>
<td><strong>Urothelial dysplasia</strong></td>
<td><strong>Recurrence</strong></td>
</tr>
<tr>
<td>Primary</td>
<td>Unknown</td>
</tr>
<tr>
<td>Secondary</td>
<td>73% vs. 43% in tumors without adjacent dysplasia</td>
</tr>
<tr>
<td><strong>Urothelial CIS</strong></td>
<td><strong>Primary</strong></td>
</tr>
<tr>
<td>Secondary</td>
<td>37%</td>
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*Recurrence or progression after BCG therapy in primary urothelial CIS. **Recurrence or progression after definitive therapy.
further subclassified into mild or moderate dysplasia. Furthermore, the 2004 WHO classification suggests that the term mild dysplasia not be used and that flat lesions with minimal cytologic atypia and architectural disorder should be recognized within the spectrum of normal urothelium.

**Immunohistochemistry**

Immunohistochemical features of urothelial dysplasia include aberrant cytokeratin 20 (CK20) expression (Figure 1) at different levels of the urothelium, but also there is usually overexpression of p53 and high Ki-67 proliferation index. While CK20 immunostaining is limited to the superficial cell layers in normal urothelium, dysplastic urothelium expresses CK20 in the superficial and intermediate cell layers. Likewise, positive CD44 immunostaining, which is observed only in the basal cell layer of normal urothelium, is found throughout the dysplastic urothelium.

**Figure 1** Urothelial dysplasia within a urothelium of reduced (A), normal (B), or increased (C) thickness, as compared to normal urothelium (D), urothelial CIS (E), flat urothelial hyperplasia (F), reactive atypia (G), and atypia of unknown significance (H). At immunohistochemistry, urothelial dysplasia expresses aberrant CK20 through all layers of the urothelium (I), variable p53 accumulation through some cells in the urothelium (<10% of cells) (J), and basal expression of CD44 (K). (A–H: hematoxylin and eosin stain; I–K: immunohistochemistry with anti-CK20 (I), anti-p53 (J), or anti-CD44 (K).)
cells in the normal urothelium, is either absent entirely or focally present in basal layers of dysplastic urothelium.\textsuperscript{24-26} In contrast, full-thickness positive membranous CD44 staining is typical of reactive urothelium. Dysplastic cells also show increased p53 expression, whereas p53 nuclear accumulation is undetectable or weak in the basal and parabasal cells of reactive urothelium (Table III).\textsuperscript{24-26} Ki-67 immunohistochemical expression is more variable since it may be seen increased in both urothelial dysplasia and reactive urothelium. The main differential consideration for urothelial dysplasia is with reactive atypia. Distinction may be particularly challenging in patients previously treated for CIS using bacillus Calmette-Guérin (BCG) intravesical immunotherapy. Immunohistochemical stains such as CK20, CD44, p53, and Ki-67 may be helpful.\textsuperscript{24-26} In this setting it is important to keep in mind that when CIS recurs, its morphology and immunohistochemistry remains similar to the untreated form. Likewise, a point of caution is the finding that molecular (multicolor fluorescence in situ hybridization [FISH] using the UroVysion probe set [Ab-\textsuperscript{bott Molecular Inc., Des Plaines, Illinois, U.S.A.] and immunohistologic (expression of CK20, high-molecular-weight cytokeratin, Ki-67, p53, and p16-INK4a) analyses cannot reliably solve diagnostic variation of flat intraepithelial lesions of the urinary bladder in some cases,\textsuperscript{27} emphasizing the fact that diagnosis of flat lesions with atypia ultimately re-

| Pathologic Features of Flat Intraepithelial Lesions Arising in the Urothelium |
|-----------------|-----------------|-----------------|-----------------|
|                  | Reactive atypia | Hyperplasia     | Dysplasia       | Urothelial CIS  |
| Cell layers      | Variable        | > 7 cells       | Variable        | Variable        |
| Polarization     | Slightly abnormal | Normal         | Slightly abnormal | Normal         |
| Cytoplasm        | Vacuolated      | Homogeneous     | Homogeneous     | Homogeneous     |
| N:C ratio        | Normal or slightly increased | Normal or slightly increased | Slightly increased | Increased       |

N:C = nucleus-to-cytoplasm ratio.

| Immunohistochemical Features of Flat-related Lesions |
|-----------------|-----------------|-----------------|
|                  | Normal          | Reactive atypia |
| CK20             | Limited to      | Limited to      |
|                  | umbrella cells  | umbrella cells  |
| CD44             | Limited to      | Increased       |
|                  | basal cells     | reactivity in all cell layers |
| p53              | Absent          | Absent          |

<table>
<thead>
<tr>
<th>Atypia of unknown significance</th>
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<tbody>
<tr>
<td>Dysplasia</td>
</tr>
<tr>
<td>Urothelial CIS</td>
</tr>
<tr>
<td>Deep layers</td>
</tr>
<tr>
<td>Absent</td>
</tr>
<tr>
<td>Absent to limited to some basal cells</td>
</tr>
<tr>
<td>Positive, frequently weak staining, usually &lt; 10% of cells</td>
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<table>
<thead>
<tr>
<th>Urothelial CIS</th>
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<tr>
<td>Positive, frequently through all cell layers</td>
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Table II

Table III
lies on histological characteristics of the lesion and the experience of the pathologist. A conservative approach with repeat cystoscopy and biopsy after the inflammation has resolved is suggested in equivocal cases.2

Recent studies show moderate to strong HER2 expression in CIS, and absent to weak HER2 expression in urothelial dysplasia and reactive conditions. From a differential diagnostic standpoint, immunostaining for HER2 protein might represent a useful adjunct to aid in the delineation between CIS and urothelial dysplasia and reactive conditions of the bladder.28 Additionally, cyclin D3 gene is amplified in urothelial CIS and not in urothelial dysplasia and reactive conditions of the bladder.29 Lewis(y) antigen expression in urothelial dysplasia and reactive conditions of the bladder (patchy discontinuous expression restricted to individual cells scattered singly throughout the urothelial mucosa) differed from urothelial CIS (full thickness expression throughout the entire urothelium including the basal cell layer) in one study.30

**Clinical Significance of Urothelial Dysplasia**

Clinically, dysplasia occurs in two distinct clinical settings: (1) de novo (primary, isolated) and (2) in patients with concurrent or previous urothelial neoplasms (secondary, concomitant).2,3,20-22 The true incidence of de novo dysplasia in the general population is unknown due to lack of large-scale screening studies. In an autopsy series of 313 cases Shirai et al15 found dysplasia in 6.8% of males and 5.7% of females. Clinical information on patients with de novo dysplasia is limited as well. Cheng et al4,20 reported 15–19% biopsy-proven cancer including CIS, papillary urothelial carcinoma and high-grade invasive carcinoma in patients with primary dysplasia. Similarly, Zuk et al21 found that 15% of patients with primary urothelial dysplasia and a mean follow-up of 4.8 years developed CIS. Secondary dysplasia is more common than primary dysplasia and has a higher rate of progression than de novo dysplasia (Table I).

**Urothelial Dysplasia: Differential Diagnosis**

**Normal Urothelium**

The urinary bladder is lined by a multilayer mucosa. The thickness of this mucosa varies according to degree of distension. Normal urothelium is usually 4–7 cells thick in a contracted bladder (Figure 1).2,22 There are 3 cell layers: basal, intermediate, and superficial umbrella. The basal cells are small and cuboidal. Their nuclei have condensed chromatin and the cytoplasm is scant. Intermediate cells are slightly larger than basal cells. The intermediate cell layer may be up to 5 cells thick. Intermediate cell nuclei are oval and have finely stippled chromatin.2,3 They have moderate amounts of cytoplasm and distinct cytoplasmic membranes. Normal intermediate cells demonstrate apical-basal polarity with the long axis perpendicular to the basement membrane. Superficial umbrella cells are large, elliptical cells with abundant cytoplasm. Umbrella cell nuclei have condensed chromatin with prominent nucleoli and occasional binucleation.2 These features should not be misinterpreted as indicating dysplasia. All 3 layers of normal urothelium typically contain glycogen, which dissolves during processing, resulting in clear areas (cytoplasmic clearing). Cytoplasmic clearing may be lost in dysplasia. Mitotic figures are usually not apparent in normal urothelium, and there is orderly maturation from basal to superficial cells. Loss of normal polarity, nuclear crowding and loss of cytoplasmic clearing with increased eosinophilia are often indicative of intraepithelial neoplasia.2,3

In normal urothelium the immunohistochemical profile of CK20, p53, Ki-67, and CD44 shows CK20 expression limited to the superficial umbrella cells.24-26 Nuclear staining for p53 is absent in normal urothelium but variably present in other flat urothelial lesions with atypia. Ki-67 is absent to positive in fewer than 10% of the basal and parabasal cells of normal urothelium, indicating a low proliferative rate. CD44 staining is limited to the basal region. Recent molecular studies have demonstrated generalized genetic instability in phenotypically normal urothelium from bladders with urothelial carcinoma.51 This observation supports the concepts of malignancy-associated changes and field change in bladder urothelial carcinogenesis. Loss of heterozygosity of chromosome 9q and mutations in FGFR3 appear important in early bladder carcinogenesis, while other gene abnormalities such DBC1, TP53 and RB1 are involved in tumor progression.17,31-34

**Flat Urothelial Hyperplasia**

Urothelial hyperplasia is characterized by markedly thickened mucosa with an increase in the number of cell layers, usually 10 or more (Figure 1).2,3 The cells do not show any significant cytologic abnormalities and retain evidence of maturation from
base to surface. Tangential sectioning of the mucosa with pseudopapillary growth may resemble flat urothelial hyperplasia. Flat urothelial hyperplasia may be associated with inflammatory disorders, urolithiasis, dysplasia, CIS and low-grade papillary tumors.\(^2,17\) There is no evidence to suggest that primary urothelial hyperplasia is premalignant. Likewise, molecular analyses have shown that this lesion may be clonally related to the papillary tumors in patients with known bladder cancer.\(^33\) Hyperplastic urothelium may be encountered within the entire spectrum of flat intraepithelial lesions with atypia (reactive atypia, dysplasia and CIS), a diagnostically challenging situation in some cases.

The true incidence of flat hyperplasia is not known due to lack of large-scale screening studies. Flat urothelial hyperplasia may occur adjacent to low-grade papillary tumors or as an isolated lesion. Isolated flat hyperplasia does not appear to have a premalignant potential. Genetic alterations in chromosome 9 are frequent in flat hyperplasia with concurrent low-grade papillary tumors.\(^17,33,34\) FISH studies for 9q22 (FACC) and 9p21 (p16/CDK12) have shown the same chromosome 9 deletions in hyperplasia and normal urothelium when there are coexisting low-grade papillary tumors. In contrast, alterations involving 17p13 (TP53) are uncommon in urothelial hyperplasia.\(^17,33,34\)

Molecular studies have also shown that flat hyperplasia adjacent to papillary tumors may show a high degree of genetic similarity to the papillary tumor cells. A possible genetic relationship between flat hyperplasia and low-grade papillary tumors has further been supported by recent molecular studies showing chromosome 9q deletions and mutations in the fibroblast growth factor receptor 3 gene in both urothelial hyperplasia and concomitant low-grade papillary neoplasia.\(^17,33,34\)

**Reactive Urothelial Atypia and Urothelial Atypia of Unknown Significance**

Reactive atypia is characterized by mild nuclear abnormalities occurring in association with acute or chronic inflammation. In most cases there is a history of cystitis, instrumentation, infection, stones, or previous therapy.\(^2\) The urothelium may be thickened and the cells are often larger than normal (Figure 1). The cytoplasmic content may be increased, imparting a squamoid appearance. Nuclei are uniformly enlarged, with vesicular chromatin and prominent centrally located nucleoli. The cells maintain polarity, and nuclear pleomorphism is generally lacking. Frequent mitoses may be present in the lower epithelial layers.\(^2\) Inflammatory cells in the lamina propria and infiltrating into the urothelium are commonly present. Diffuse CK5/6 reactivity in reactive urothelial atypia and negative CK5/6 reactivity in urothelial CIS may be helpful in distinguishing between these 2 entities, as seen in a recent study.\(^35\)

The term *atypia of unknown significance* was introduced by the ISUP consensus group to describe lesions for which there was uncertainty about whether the changes were reactive or dysplastic.\(^3\) The degree of nuclear polymorphism and hyperchromasia present is greater than that in reactive atypia, and dysplasia cannot be ruled out definitively. The cellular changes present are disproportionate to any degree of inflammation seen. There is no evidence supporting a premalignant nature of such lesions at the present time, and the use of the designation *atypia of unknown significance* is discouraged.\(^2,4\)

Overall, CK20 and CD44 appear to be the most useful objective markers for distinguishing reactive atypia/atypia of unknown significance from dysplasia.\(^26\) As mentioned previously, CK20 expression is normally limited to the superficial umbrella cells. Reactive urothelium typically shows staining with CD44 in a diffuse, membranous, full-thickness pattern or with patchy basal and intermediate cell expression. This is in contrast to absence or the presence of limited reactive cells in basal layers of CD44 staining in dysplasia.\(^26\) It is important to note that these staining patterns are not absolute. Therefore, caution must be exercised when interpreting them, and clinical correlation with morphology is critical.

**Urothelial CIS**

Urothelial CIS, also known as *high-grade intraurothelial neoplasia*, is a flat lesion characterized by the presence of unequivocal cytologically malignant cells, some of which may be anaplastic (Figure 1).\(^36,37\) Full thickness cytoplastic atypia is not required for the diagnosis.\(^2,3\) The urothelium may be denuded, reflecting the discohesive nature of the cells, or it may be diminished in thickness, of normal thickness, or hyperplastic. Superficial (umbrella) cells may or may not be present. Marked disorganization of cells with loss of cellular polarity and decreased cellular cohesiveness is a common feature.\(^2\) The tumor cells tend to be large and pleomorphic, with moderate to abundant cytoplasm,
coarse and clumped chromatin, and multiple prominent nucleoli. Atypical mitotic figures may be present, often extending to the upper layers of the urothelium. The adjacent mucosa often contains lesser degrees of cytologic abnormality. The lamina propria is frequently hypervascular and inflamed. Nucleomegaly is a common finding in CIS, and it is determined by comparison to normal urothelium and stromal lymphocytes.

Large nuclei in CIS are about 5 times the size of a normal lymphocyte, whereas the nuclear size of normal urothelium was approximately twice the size of lymphocytes.

Additionally, there are some morphologic variants of urothelial CIS. These include large-cell CIS, small-cell CIS, denuding and clumping pattern with discohesive cells, lepidic or undermining pagetoid CIS, microinvasive CIS, and CIS with squamous or glandular differentiation. The high content of pleomorphic cells in some CIS cases argues in favor of a giant cell variant of CIS. Awareness of the histologic diversity of CIS may aid in differential diagnosis.

Urothelial CIS shows intense aberrant CK20, increased Ki-67 labeling, and p53 positivity in the majority of malignant cells. The neoplastic cells are uniformly negative for CD44 immunostaining.

**Therapy-induced Reactive Changes in the Urothelium**

Several systemic or intravesical antineoplastic agents, such as thiotepa (triethylenethiophosphoramide), mitomycin C, cyclophosphamide, ketamine, BCG, and radiation therapy, produce atypical urothelial changes that can mimic dysplasia or cancer histologically. Reactivation of polyomavirus infection can also be an important mimic of urothelial dysplasia or CIS.

Mitomycin C and thiotepa induce exfoliation, epithelial denudation, multinucleation, cytoplasmic vacuolization, and the appearance of bizarre, nonmalignant nuclei in the superficial layers of the urothelium. A marked necroinflammatory process with a histiocytic response extending deep into the bladder wall and eosinophilic cystitis may be seen with mitomycin C. In addition, both mitomycin C and thiotepa destroy the tips of the papillae of papillary urothelial carcinoma. These truncated papillae, when associated with denudation and inflammation, may be mistaken for CIS or dysplastic changes instead of residual papillary urothelial carcinoma.

Cyclophosphamide therapy may induce stromal fibrosis, vascular intimal thickening, mural fibrin deposition in vessels, vascular ectasia, and epithelial necrosis. Regenerative changes with binucleated and multinucleated cells, often with large, bizarre nuclei, may be mistaken for malignancy. Cyclophosphamide may also induce reactivation of polyomavirus infection, causing marked nuclear atypia in the surface urothelium, a lesion that mimics urothelial dysplasia/CIS. Patients receiving ketamine may present reactive urothelial changes that can mimic urothelial dysplasia/CIS.

In BCG-treated bladders, reactive, mostly reparative changes may develop as a result of acute and chronic inflammation, reactive epithelial atypia, and granulomatous reaction deep in the bladder wall. It is important to keep in mind that CIS may recur after BCG therapy and that this may be seen mainly in Brunn nests.

Radiation therapy produces a variety of bladder lesions beginning with acute cystitis and hyperemia with edema of the lamina propria approximately 3–6 weeks after therapy. The urothelium shows cytoplasmic and nuclear vacuolization, karyorrhexis, stromal hyalinization, thrombosis of blood vessels, stromal cell atypia, and fibroblast proliferation. Bizarre, multinucleated cells with enlarged nuclei, smudged chromatin, and degenerative changes are usually present in radiation atypia and are not seen in dysplasia/CIS. Pseudocarcinomatous epithelial hyperplasia, a lesion seen occasionally after radiation and/or chemotherapy, should be distinguished from flat lesions with atypia.

Pathologists should be aware of these diagnostic pitfalls and exercise caution when evaluating urothelial atypia following treatment. If a distinction between treatment-induced atypia and dysplasia/CIS cannot be made, a conservative approach with follow-up repeat biopsy is indicated after inflammation has subsided.

**Conclusion**

Flat urothelial lesions with atypia comprise a spectrum of morphologic changes ranging from reactive atypia to urothelial dysplasia or CIS. Differentiating these lesions is important because of different clinical outcome and therapy. Currently, differential diagnosis relies on histopathological evaluation of samples and the experience of the pathologist. Combined use of CK20, p53, and CD44 may allow discrimination between reactive and neoplastic flat lesions in difficult cases.
References


