Introduction

The etiology and pathogenesis of human transitional cell carcinoma (TCC) remain unknown at the present time. While many carcinogens can induce bladder carcinoma in animal experimental studies, the only well-established carcinogens in human beings are tobacco smoke and arylamines.[1] Experimental studies in animals have demonstrated that invasive urothelial carcinoma is preceded by a sequence of progressive intraepithelial changes: generalized thickening of the urothelium or hyperplasia, disordered arrangement of cells with nuclear atypia or dysplasia, and fully developed carcinoma in situ (CIS).[2]

The concept of a continuum biologic spectrum of intraepithelial abnormalities preceding invasive carcinoma is well established in cervical cancer. The term "cervical intraepithelial neoplasia" (CIN) was coined to embrace this spectrum. The homologous term "urothelial intraepithelial neoplasia" (UIN) has been used by some authors to advocate a similar pathogenesis in bladder cancer. However, the validation of this concept in humans is controversial, and the term UIN has not been widely accepted. Many investigators dislike the concept of continuum biologic spectrum when applied to bladder cancer, and some claim that the progression from lesser to more malignant urothelial lesions might be unusual. In the following discussion, urothelial dysplasia and CIS are reviewed as two distinct forms of preinvasive urothelial neoplasia.

Urothelial Dysplasia

Experimental animals treated with carcinogens develop dysplasia prior to TCC.[2] However, this development is not well established in human beings because urothelial dysplasia is asymptomatic, it cannot be identified cystoscopically, and it sheds few or no cells into the urine. As a result, the great majority of urothelial dysplasia are discovered in patients who already have developed TCC. The frequency of this association varies from 20% to 80%, depending on the thoroughness of the search.[3] When dysplasia is associated with TCC, the risk of recurrence and progression is apparently increased.

In both concept and definition, dysplasia in the urothelium differs from dysplasia in the uterine cervix. The definition and diagnosis of urothelial dysplasia comprise a spectrum of intraepithelial changes that develop in flat urothelium. While the changes associated with dysplasia are easily distinguished from CIS, they are more difficult to distinguish from changes in some of the reactive or reparative processes.

The morphologic features of dysplastic cells are similar to those in transitional cell papillomas (papillary TCC grade I of the World Health Organization), but they occur in flat urothelium. When evaluating a bladder biopsy for urothelial dysplasia or CIS, appropriate fixation is mandatory. With formalin fixation, small variations in the pH can obscure subtle cytologic abnormalities. Nucleoli and chromatin patterns may not be apparent. Bouin's or Hollande's solution provides optimal nuclear details.[4] At low magnification, areas of dysplasia are recognized by nuclear clustering, an essential diagnostic criterion.[1,5] Dysplastic cells are larger and lack the normal cytoplasmic clearing.[1,5] The nuclear:cytoplasmic ratio is increased, and nuclear pleomorphism and nuclear border irregularities (eg, shallow depressions and nonprominent creases or notches) are present (Fig 1). The differences between CIS cells and dysplastic cells are so apparent that the differential diagnosis is rarely a problem. Pronounced nuclear notching or creasing is not observed in dysplastic cells.

The chromatin in dysplastic cells is fine and regular, and the nucleoli are either small or absent. Mitoses are present but not numerous.

There is a spectrum of variability in the manifestation of these cellular changes (Table). Dysplasia can be graded as mild or moderate, but this grading lacks any practical application. The differential diagnosis, however, can be more difficult to establish. The nucleoli of reactive and reparative urothelial cells may be enlarged, but they lack the nuclear border irregularities of dysplastic cells, they retain cytoplasmic vacuolization, and they are prominent with fine nuclear chromatin.[1,5] Intraurothelial inflammatory cells, if present, favor a reactive process. The large and closely packed urothelial cells of the bladder neck may resemble dysplasia, but their nuclei are evenly distributed and lack nuclear border irregularities. Urothelial artifacts such as attenuation or compression (eg, lymphoid follicles, von Brunn's nests) result in abnormal cytologic features that resemble dysplasia. Careful attention is mandatory when evaluating urothelium affected by these artifacts.

<table>
<thead>
<tr>
<th>Cytologic Features</th>
<th>Dysplasia</th>
<th>Carcinoma in Situ</th>
<th>Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear atypia</td>
<td>Mild to moderate increase</td>
<td>Marked increase</td>
<td>Mild increase</td>
</tr>
<tr>
<td>Cytokinesis</td>
<td>Lack cytoplasmic clearing</td>
<td>Marked cytoplasmic clearing</td>
<td>Retain cytoplasmic clearing</td>
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<tr>
<td>Nuclear borders</td>
<td>Shallow depressions</td>
<td>Deep depressions</td>
<td>Prominent creases</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Finely granular</td>
<td>Coarsely granular</td>
<td>Finely granular</td>
</tr>
<tr>
<td>Nucleoli</td>
<td>Inconspicuous</td>
<td>Large in many cells</td>
<td>Prominent in all cells</td>
</tr>
<tr>
<td>Mitoses</td>
<td>Sparse, present but abnormal</td>
<td>Many present and some abnormal</td>
<td>Few (plump) and normal</td>
</tr>
</tbody>
</table>
Although experimentally induced dysplasia can progress to CIS and invasive cancer, the biologic behavior of urothelial dysplasia and CIS in human beings remains controversial.

**Urothelial Carcinoma In Situ**

Urothelial cancer progresses in either papillary or flat architectural patterns. Although noninvasive papillary TCC is a CIS, it is excluded from the definition of urothelial CIS and is not addressed here. In situ lesions of squamous or glandular differentiation may be seen, but they are unusual, are not well defined, and are excluded from CIS definition. Thus, urothelial CIS is defined as a flat, intraurothelial neoplasm with high-grade nuclear features.

Nonselective urothelial abnormalities may progress to CIS but, in most cases, CIS arises de novo. Two clinical types of CIS are secondary and primary CIS. Most instances of CIS are associated with concomitant invasive TCC in adjacent or distant urothelium (secondary CIS). Occasionally, however, CIS is found without associated invasive TCC (primary CIS). Given the rarity of primary CIS in human beings (1% or less), the investigation of the biologic behavior of CIS in vivo is difficult and controversial. Many reports in the literature describe urothelial CIS as a highly aggressive malignant neoplasm. However, in one study,6 the mortality rates of primary CIS and secondary CIS were 6.9% and 45.2%, respectively, suggesting that many instances of CIS fail to progress to invasive carcinoma.

The clinical features of primary CIS are nonspecific. Gross or microscopic hematuria is the most common feature, but this condition is often nonspecific (e.g., cystitis). Endoscopically, the lesion is difficult to localize and appears as erythema or urothelial roughening.

While urothelial CIS is easily recognized in cytologic specimens, it is often difficult to confirm histologically due to weak tumoral cell cohesion and easy exfoliation of large numbers of cells with demudation of the urothelium.[1,5] An adequate specimen contains several layers of anaplastic cells, particularly if the patient has not been previously diagnosed or treated for CIS. Contrary to cervical CIS, however, full-thickness anaplasia is not required for the diagnosis of urothelial CIS.[1,5] In fact, the surface of CIS often contains mature urothelial cells. CIS involvement is usually well demarcated from the adjacent mucosa. The most important diagnostic criterion is the presence of malignant urothelial cells with high-grade nuclear features, regardless if present individually, in small groups, or throughout the full thickness of the urothelium.[1,5]

Unlike papillary TCC, hyperplasia is not a common component of CIS. The high-grade features consist of marked nuclear shape, pleomorphism, and nuclear border irregularities (e.g., prominent nuclear notching and creasing with little variation in nuclear size).[1,5] These features correlate with the morphology of high-grade papillary TCC (most of grade II and all of grade III TCC of the World Health Organization). The chromatin is coarsely granular, and the nucleoli are large (although not in every cell). Mitoses are variable, occasionally abnormal, and occur more often compared to dysplasia. The degree to which these features are manifested among CIS is variable, but there is no current evidence that this variability has any biologic significance, and grading is not recommended.

Histologically, several variants of CIS are recognized. The large-cell variant[1,5] is the most common. The tumor cells are easily recognized at low magnification because they are larger and more amphiphilic than normal urothelial cells. The nuclear:cytoplasmic ratio is increased, the nuclei are eccentric with occasional overlapping, and the morphologic features described earlier can be seen (Fig 2). The pagetoid variant[7] features isolated or small clusters of large eosinophilic cells that resemble extramammary Paget's disease and are scattered throughout normal urothelium (Fig 3). The cells have a low nuclear:cytoplasmic ratio but marked nuclear anaplasia and prominent nucleoli. This variant is always observed near invasive carcinoma and is never a primary CIS. The small-cell variant[1,5] is a rare form of CIS that is difficult to recognize at low magnification and easy to overlook. However, the small-cell variant is usually hyperplastic, a feature rarely observed in CIS and a clue to its diagnosis (Fig 4). The cytologic features are not significantly different from the large-cell variant.

Urothelial CIS tends to spread intraepithelially to normal adjacent mucosa, which explains its frequent extension to prostatic ducts and occasional extension to distal ureters.[1,5] Since urothelial CIS is composed of high-grade malignant cells, it is erroneously assumed that they always pursue a malignant clinical course. While this might be true for secondary CIS, only one third of primary CIS invades the lamina propria or muscle wall, and the mortality rate is only 7% to 20%.6

Commonly underlying any histologic variant of CIS are extensive lymphocytic infiltrates, probably representing a host immune response to the tumor. Based on this observation and to explain the low frequency of invasion in primary CIS, it has been hypothesized that urothelial CIS is the remnant of a neoplasm with an aborted invasive potential by host immune responses.[6]

The use of urine cytology to diagnose either urothelial dysplasia or low-grade papillary TCC presents the same difficulties. The sensitivity is low because dysplastic cells rarely shed to the urine (Fig 5). In contrast, the diagnostic sensitivity of CIS is higher by urine cytology than by true biopsy (Fig 6).
Molecular Aspects of Urothelial Dysplasia and Carcinoma In Situ

ABH blood-group antigens are commonly expressed by normal urothelial cells. However, ABH antigenic expression is abnormal in urothelial dysplasia and CIS.[8] A gradient of antigenic abnormalities is often observed when normal urothelium, dysplastic urothelium, and CIS are compared. ABH antigenic urothelial expression can be demonstrated by immunohistochemistry. Immunostaining of bladder biopsies from patients with the same ABH blood group who are diagnosed with dysplasia or CIS has revealed that, compared with normal urothelium, the immunostaining reaction is decreased in dysplastic cells and is weak or negative in CIS. Also, in patients with bladder cancer, histologically normal urothelium can demonstrate antigenic abnormalities similar to those found in urothelial dysplasia or CIS. Based on these observations, a histologically benign but antigenically abnormal urothelium has been postulated to contain the incipient abnormalities of a low-grade CIS.[8]

Two genetic alterations -- loss of chromosome 9 heterozygosity and p53 gene mutations -- may affect urothelial tumorigenesis and may also represent two distinct pathways of tumor progression. Loss of chromosome 9 heterozygosity is observed in 34% of papillary TCC but in only 12% of urothelial dysplasia and CIS.[9] Mutations in the p53 gene, however, are observed in 65% of urothelial dysplasias and CIS but in only 3% of papillary TCC.[9] The frequency of p53 gene mutations in urothelial dysplasia and CIS is similar to its frequency in muscle-invasive TCC (51%). This may explain the propensity of these lesions to progress to invasive cancer since these mutations are known to destabilize the genome. This may allow the accumulation of a sufficient number of gene mutations in CIS to invade the muscle wall and to metastasize. Mutations in the p53 gene can be detected in tissue sections by immunohistochemistry. Since the wild-type p53 gene has a short life, immunostaining of normal urothelium with p53 monoclonal antibodies is negative. When mutations in the p53 gene occur, the mutated proteins aggregate in tetrameric and pentameric macromolecules of longer life. The result is an accumulation of p53 protein that provides a positive immunostaining reaction. The reaction is observed in the nuclei of tumor cells affected by these events. The role of the immune system in neutralizing the invasive potential of CIS is unknown but may explain why a significant proportion of CIS fails to invade.

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References


