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Immunohistochemistry in Urothelial Dysplasia

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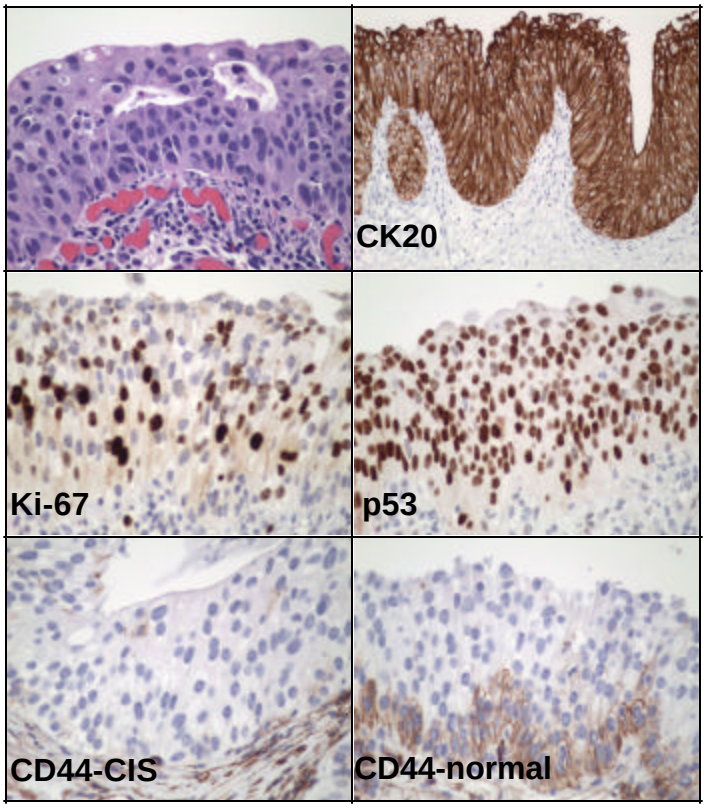
by Rodney T. Miller, M.D., Director of Immunohistochemistry

Urothelial dysplasia is a fairly common problem, and many surgical pathologists are called upon to review bladder biopsies to assess the presence or absence of urothelial dysplasia or carcinoma in situ (CIS). The accurate recognition of CIS is important for appropriate patient management, since those with CIS are at increased risk of invasive urothelial carcinoma. Unfortunately, there are cases that are very difficult to interpret on H&E, where distinguishing reactive changes from true dysplasia or CIS is problematic. This month, we review two published papers describing the use of selected immunostains to assist in the accurate recognition of urothelial dysplasia and CIS.



H&E (top left) and immunostains from a case of urothelial high grade dysplasia/CIS. Note aberrant full thickness expression of CK20 (top right), increased Ki-67 positive cells (left middle) and strongly positive p53 (middle right). CD44 is negative in CIS (bottom left), in contrast to the typical basal layer staining pattern of normal urothelium (bottom right).

In a paper published in the American Journal of Surgical Pathology in August 2001, McKenney and associates from Emory University described the use of immunostains for cytokeratin 20 (CK20) (a cytoplasmic antigen), p53 (a nuclear antigen), and the standard isoform of CD44 (expressed on the cell membrane) to address this problem. Their study included 21 cases of CIS, 15 urothelial biopsies with reactive atypia, and 10 normal ureters from nephrectomy specimens. "Overexpression" of p53 and CK20 were defined as follows: ">50% of the urothelium had to be moderately to strongly positive". In their study, they noted distinctly different patterns of expression of these markers in normal urothelium, reactive atypia, and CIS. In normal urothelium, CK20 showed patchy expression only in the umbrella (superficial) cell layer of the epithelium, CD44 showed patchy membranous staining of the basal layer in all cases, and p53 was negative or only focally weakly expressed (<10% of cells, weak expression). Cases of reactive atypia showed similar CK20 reactivity to normal urothelium (apparent only in the umbrella cell layer), and p53 was at most patchy and weak, mostly in the basal cell layer.

However, immunostains for CD44 showed membranous expression in the full thickness of the epithelium in 60% of cases of reactive atypia, with the other 40% of cases showing patchy positivity in the basal or intermediate cell layers, similar to the findings noted with normal urothelium. The strongest full thickness reactivity with CD44 was noted in those cases of reactive atypia in which the cytologic changes were most striking. In contrast, cases of CIS showed overexpression of CK20 (defined as > 50% of neoplastic cells) in 81% of cases, and p53 was overexpressed in 57% of the cases of CIS. In contrast to both normal urothelium and reactive atypia, CD44 immunoreactivity was not observed in cases of CIS. In some of these cases however, a residual non-neoplastic basal cell layer was present beneath the CIS, that did show expression of CD44. In one of the cases of pagetoid CIS, CK20 and p53 stained only the neoplastic cells within the adjacent benign reactive epithelium, although the benign reactive epithelium showed strong membrane reactivity with CD44, as might be expected.

In the March 2003 issue of Modern Pathology, Mallofre and associates from the University of Barcelona performed a similar study employing CK20, p53, and Ki-67. Their study included 50 cases of morphologically clear-cut CIS, 40 non-neoplastic urothelial biopsies, and 30 cases showing atypia of indeterminate significance. Ki-67 was considered "positive" when >10% of cells showed nuclear reactivity, and p53 was considered "positive" when >20% of cells showed nuclear reactivity. Aberrant CK20 expression was defined as reactivity in urothelial cells other than superficial umbrella cells. Non-neoplastic urothelium showed reactivity for CK20 only in umbrella cells, and p53 and Ki-67 were negative or only weakly reactive in <10% of basal cells. In the cases of CIS, Ki-67 was positive in 94% of cases, with 38% of cases showing >50% positive cells. 80% of cases showed positive p53, and 72% showed aberrant CK20 reactivity. In the group of cases showing atypia of indeterminate significance, 73% were positive for p53, 40% for Ki-67, and 30% for CK20 (23% of cases were negative for all antibodies). The authors concluded that this antibody panel was helpful in distinguishing dysplastic changes from reactive atypia, and found that it was particularly useful for confirming CIS in cases with denuded epithelium or in cases of small cell CIS.

All of these markers are available in the ProPath Immunohistochemisty Lab for those who want to make use of them for their diagnostically challenging bladder biopsies.

A table summarizing the expected results is listed below.

	Normal	Reactive	CIS
CK20	umbrella only	umbrella only	Aberrant (70-80%)
p53	neg/foc weak	neg/foc weak	Pos (60-80%cases)
CD44	basal only	full/partial thickness	CIS cells neg
Ki67	<10% cells		>10% cells in 90% cases

References:

1.McKenney JK et al: Discriminatory Immunohistochemical Staining of Urothelial Carcinoma In Situ and Non-Neoplastic Urothelium. An Analysis of Cytokeratin 20, P53, and CD44 Antigens. American Journal of Surgical Pathology 25 (8): 1074-1078, 2001.

2. Mallofre C et al: Immunohistochemical Expression of CK20, P53, and Ki-67 As Objective Markers of Urothelial Dysplasia. Modern Pathology 16:187-191, 2003.

Rodney T. Miller, M.D.
Director of Immunohistochemistry
214-237-1631 • Fax 214-237-1770
rmiller@propathlab.com

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PROPATH
8267 Elmbrook Drive, Suite 100 • Dallas, Texas 75247-4009
214-638-2000 • Toll Free: 800-258-1253 • Fax: 214-905-3457
www.propathlab.com