CD10 Immunohistochemical Staining in Urothelial Neoplasms

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Abstract

CD10, or common acute lymphoblastic leukemia antigen (CALLA), is a cell surface neutral endopeptidase that inactivates various bioactive peptides.1,2 It is expressed by hematopoietic cells and their neoplasms, ie, acute leukemias and lymphomas.1 CD10 has also been demonstrated in a variety of nonhematopoietic tissues and neoplasms.1 Koiso et al3 suggested that CD10 might serve as a useful marker for urothelial carcinoma and showed differential staining intensities in superficial and invasive urothelial carcinoma. Chu and Arber1 studied CD10 staining in 24 cases of urothelial carcinoma and found positive staining in 54% of the cases. Other studies have shown absent staining in normal urothelium4 and low levels of staining in invasive urothelial carcinoma.5 To our knowledge, a comparative study of the CD10 immunoprofile in a range of urothelial tumors has not been performed. We performed CD10 immunohistochemical staining on a spectrum of urothelial lesions representing diagnostic entities as defined by the 2004 World Health Organization classification of urothelial neoplasia.6

Materials and Methods

We obtained 70 bladder biopsy and cystectomy specimens from the files of Douglass Hanly Moir Pathology, Macquarie Park, Australia, representing 10 cases each (diagnosed in 2003) of the following diagnostic categories: normal or reactive urothelium (NEG), papillary urothelial neoplasm of low malignant potential (PUNLMP), low-grade papillary urothelial carcinoma (LGPUC), urothelial dysplasia (DYSP), carcinoma in situ (CIS), high-grade papillary urothelial carcinoma (HGPUC), and infiltrating urothelial carcinoma (IUC).
The tissue samples from each case had been fixed in 10% neutral buffered formalin and embedded in paraffin. We placed 4-µm sections of the formalin-fixed, paraffin-embedded index tissue, as well as sections of control tissue (tonsil and small bowel mucosa), on glass slides, which were dried for 2 hours at 65°C before being dewaxed in xylene and hydrated via graded alcohols. Epitope retrieval was performed using a citrate-EDTA solution (pH 7.6) heated from room temperature to 98°C during a 15-minute period in a microwave and held at that temperature for another 10 minutes before being allowed to cool to room temperature. A standard streptavidin-biotin method using horseradish peroxidase and diaminobenzidine was performed on a Bond X autostainer (Vision Biosystems, Mount Waverly, Australia) using a monoclonal mouse CD10 antibody (clone 56C6, catalog No. NCL-CD10-270; Novocastra, Newcastle upon Tyne, England) at a 1:80 dilution, incubated for 15 minutes at room temperature. All other incubations used the Bond Peroxidase Detection System with counterstain (catalog No. DS 9404; Vision Biosystems). Sections then were dehydrated with graded alcohols, cleared in xylene, and coverslipped.

We evaluated the stained sections in random order; we were blinded to the diagnoses. Brown staining of the cell membrane and/or cytoplasm was considered positive. The intensity of staining was graded on a scale of 0 (absent) to 3 (strong), compared with the control tissue samples on the slide, and the distribution of staining (superficial, deep, or superficial and deep, ie, diffuse) was evaluated in all cases except invasive carcinoma. Statistical significance of the results was evaluated using the χ² test.

**Results**

We demonstrated positive CD10 staining in 64 (91%) of 70 cases. There was moderate to strong CD10 staining (intensity, 2 or 3) in 45 (64%) of 70 cases; of these, 10 cases represented nonneoplastic urothelium (NEG), and 5 (50%) of the 10 cases showed positive CD10 staining [Table 1, Table 2, Figure 1, and Figure 2]. The diagnostic categories studied showed varying intensity of staining with CD10. In general, the staining intensity for IUC, HGPUC, and CIS cases was high, whereas LGPUC, PUNLMP, and NEG cases showed low staining intensity, and DYSP cases showed intermediate staining intensity [Image 1]. These findings reached statistical significance when the mean staining intensities of IUC, CIS, and HGPUC were compared with those for LGPUC and NEG and when the staining intensities for CIS and HGPUC were compared with the intensity for PUNLMP. When the cases were grouped, there was a statistically significant difference in the staining intensity, with the IUC-CIS-HGPUC group showing higher mean scores than the LGPUC-PUNLMP-NEG group, and the DYSP cases showing intermediate scores [Table 3].

The distribution of CD10 staining was assessed in all cases except IUC and categorized as absent, deep, superficial, or deep and superficial (ie, diffuse). The results within each diagnostic category are summarized in [Table 4] and [Figure 3]. Of the cases that stained positively with CD10, there was a trend toward more diffuse staining distribution in the higher grade lesions (HGPUC and CIS), whereas the lower grade lesions (LGPUC, PUNLMP, and NEG) showed diffuse staining.

**Table 1**

<table>
<thead>
<tr>
<th>Intensity</th>
<th>IUC</th>
<th>CIS</th>
<th>HGPUC</th>
<th>DYSP</th>
<th>LGPUC</th>
<th>PUNLMP</th>
<th>NEG</th>
<th>Total</th>
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<td>1.4</td>
<td>1.2</td>
<td>1.4</td>
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| CIS, carcinoma in situ; DYSP, urothelial dysplasia; HGPUC, high-grade papillary urothelial carcinoma; IUC, infiltrating urothelial carcinoma; LGPUC, low-grade papillary urothelial carcinoma; NEG, normal or reactive urothelium; PUNLMP, papillary urothelial neoplasm of low malignant potential. |

*P < .05 for invasive carcinoma vs LGPUC and NEG; CIS vs LGPUC, PUNLMP, and NEG; and HGPUC vs LGPUC, PUNLMP, and NEG.

**Table 2**

<table>
<thead>
<tr>
<th>Intensity</th>
<th>IUC</th>
<th>CIS</th>
<th>HGPUC</th>
<th>DYSP</th>
<th>LGPUC</th>
<th>PUNLMP</th>
<th>NEG</th>
<th>Total</th>
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<td>4</td>
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<td>9</td>
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<td>6</td>
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<td>3</td>
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</tbody>
</table>

CIS, carcinoma in situ; DYSP, urothelial dysplasia; HGPUC, high-grade papillary urothelial carcinoma; IUC, infiltrating urothelial carcinoma; LGPUC, low-grade papillary urothelial carcinoma; NEG, normal or reactive urothelium; PUNLMP, papillary urothelial neoplasm of low malignant potential.

*Data are given as number of cases.
in approximately half of the cases; the majority of the remaining cases in these categories showed superficial staining only (LGUPC and NEG) or deep staining only (PUNLMP). When the cases were grouped, these trends became more apparent Table 5 and Figure 4. The differences between groups were statistically significant.

**Discussion**

CD10 is a membrane-associated enzyme also known as neutral endopeptidase (NEP), enkephalinase, and CALLA. NEP initially was extracted from the brush border of proximal renal tubules in rabbits in 1974. CALLA initially was identified serologically in 1975 and was assigned the cluster of differentiation number 10 (CD10) in 1984. Studies in the early 1980s showed that enkephalinase A, an enzyme found in brain tissue, was identical to NEP. The first monoclonal antibody (J-5) to CD10 was generated by Ritz et al in 1980, and a monoclonal antibody (clone 56C6) suitable for use in paraffin-embedded sections was described in 1999.

CD10 initially was identified as a cell surface antigen expressed by acute lymphoblastic leukemias, hence its early designation as CALLA. It also is expressed by other hematopoietic malignant neoplasms such as lymphoblastic lymphoma, follicular lymphoma, Burkitt lymphoma, and chronic myeloid leukemia in blast crisis. Initially, CD10 was thought to be a tumor-specific antigen, but studies have shown that it is expressed by a variety of nonneoplastic cells, including renal proximal tubular epithelial cells, early lymphoid progenitors in bone marrow and thymus, neutrophils, bronchial epithelial cells, fibroblasts, myoepithelial cells of breast and salivary glands, biliary canaliculi, intestinal epithelial cells, endometrial stromal cells, decidual cells, trophoblastic cells, and mesonephric remnants. Several nonhematopoietic tumors also show CD10 staining, such as endometrial stromal tumors, gestational trophoblastic disease, mesonephric tumors, some mesenchymal tumors, malignant melanoma, carcinomas of the lung, colon, kidney, prostate, and urothelium, and hepatocellular carcinomas.

CD10, which maps to chromosome 3q21-27, belongs to a family of peptidases that includes CD13 and CD26. It is a 90- to 110-kd cell surface glycoprotein metalloenzyme that uses zinc as a cofactor and modulates cellular responses to peptide hormones by regulating local peptide concentrations. CD10 hydrolyses a variety of peptides, including substance P, atrial natriuretic factor, endothelin, neurotensin, oxytocin, bradykinin, angiotensins, bombesin-like peptides (BLPs), calcitonin gene–related peptide, and enkephalins. It generally reduces cellular responses to peptide hormones by hydrolyzing them, thus reducing the concentration of peptide available for receptor binding and signal transduction.
In addition to its enzymatic function, the CD10 protein has a direct role in signal transduction pathways that regulate cell growth and apoptosis. CD10 has many biologic effects, including attenuation of the neutrophil inflammatory response, modulation of atrial natriuretic factor–mediated hypotension and diuresis, and reduction of enkephalinergic responses. In hematopoietic tissues, CD10 regulates early B-cell development by inactivating peptides that stimulate B-cell proliferation and differentiation. It also has been implicated in the promotion of early T-cell development and in the modulation of thymocyte and thymic epithelial cell proliferation. CD10 also regulates peptide-mediated cell proliferation in nonhematopoietic tissues, as described later.

Koiso et al were the first to assess CD10 (NEP) by biochemical assay and immunohistochemical staining in bladder cancer cells. They found that the enzyme activity and immunohistochemical staining were high in superficial bladder cancer but low in invasive bladder cancer. McIntosh et al, in the initial description of the monoclonal antibody to CD10 suitable for paraffin-embedded tissue samples (clone 56C6), tested their antibody in a variety of normal tissue samples; they reported absence of CD10 staining (cytoplasmic or membranous) in nonneoplastic ureteric transitional epithelium. Chu and Arber studied CD10 staining in a variety of normal and neoplastic nonhematopoietic tissue samples. Membranous, cytoplasmic, or apical surface staining was considered positive. Positive cytoplasmic CD10 staining was seen in 13 (54%) of 24 cases of transitional cell (urothelial) carcinoma (presumably invasive, although this is not stated in the report). Kim and Kim studied CD10 staining among various renal neoplasms.
epithelial tumors, including 25 cases of urothelial carcinoma (presumably invasive, although this is not stated in the report) of the renal pelvis and found only focal staining (≤10% of cells) in 8 (32%) of 25 cases. However, in this study, staining was considered positive only if membranous staining was seen in at least 10% of cells, which explains their low proportion of positively staining cases; in our cases of IUC, we found

**Table 4**

### Distribution of Staining

<table>
<thead>
<tr>
<th>Intensity</th>
<th>CIS</th>
<th>HGPUC</th>
<th>DYSP</th>
<th>LGPUC</th>
<th>PUNLMP</th>
<th>NEG</th>
</tr>
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<tbody>
<tr>
<td>Absent staining</td>
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<td>0</td>
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<td>1</td>
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<td>1</td>
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<tr>
<td>Superficial and deep</td>
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<td>10</td>
<td>6</td>
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<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Deep</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Superficial</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

CIS, carcinoma in situ; DYSP, urothelial dysplasia; HGPUC, high-grade papillary urothelial carcinoma; LGPUC, low-grade papillary urothelial carcinoma; PUNLMP, papillary urothelial neoplasm of low malignant potential; NEG, normal or reactive urothelium.

*P < .05 (χ² test).

CD10 immunostaining in low-grade papillary urothelial carcinoma (E, ×200), papillary urothelial neoplasm of low malignant potential (F, ×200), and normal or reactive urothelium (G, ×200).
that the predominant staining pattern was not membranous but cytoplasmic, a finding also reported by Chu and Arber.¹

We demonstrated moderate to strong CD10 staining in 40 (67%) of 60 of our cases of urothelial lesions and in 5 (50%) of 10 cases of nonneoplastic urothelium. Of 10 cases of IUC, 8 (80%) stained positively, which compares with the 54% reported by Chu and Arber.¹

The staining intensities for IUC, HGPUC, and CIS cases were higher than those for DYSP cases, which, in turn, were higher than those for LGPUC, PUNLMP, and NEG cases. These differences parallel currently understood pathways of bladder tumorigenesis. The World Health Organization's most recent classification of urothelial neoplasms⁶ stratifies these tumors into prognostically significant groups.³⁵ Two major genetic subtypes of urothelial neoplasms exist and correspond to morphologically defined entities. The genetically stable category includes low-grade, noninvasive papillary tumors (LGPUC and PUNLMP)⁶,³⁷ and is associated with a high rate of recurrence (50%-70%) but a low rate of progression (<5%).³⁵,³⁸-⁴⁰ The genetically unstable group includes HGPUC, CIS, and IUC.³⁶,³⁷ HGPUCs often show infiltration of the lamina propria, are associated with flat DYSP and/or CIS, and show a much greater risk of progression (30%-50%) to IUC. Additional genetic events may induce progression of low-grade tumors to high-grade papillary lesions but are uncommon; more often, HGPUC is thought to derive from DYSP and/or CIS via a process of papillary hyperplasia. Alternatively, DYSP might progress to flat CIS, which, in turn, might evolve into IUC. Thus, IUCs most likely originate from flat CIS or HGPUC rather than from low-grade papillary tumors.³⁵,⁴¹,⁴²

It seems that PUNLMP/LGPUC and HGPUC/CIS/IUC form distinct groups. This is the basis for the division of our cases into low-grade and high-grade diagnostic groups; the NEG cases were added to the low-grade group, given the similarity of their patterns of CD10 staining; the DYSP cases were analyzed separately because their pattern of CD10 staining seemed intermediate between the low- and high-grade groups. Analysis of the staining intensity in the IUC-HGPUC-CIS (mean, 2.5), DYSP (mean, 1.7), and LGPUC-PUNLMP-NEG (mean, 1.3) groups showed statistically significant differences. Similarly, there were statistically significant differences in the distribution of staining in the 3 diagnostic groups: the high-grade (HGPUC-CIS) group showed predominantly diffuse staining; the low-grade (LGPUC-PUNLMP-NEG) group showed a mixture of diffuse, superficial only, and deep only staining; and the DYSP group showed...
showed intermediate staining distribution (although more similar to the low-grade group).

Our findings raise several possibilities about the role of CD10 in urothelial tumorigenesis. The initial event in urothelial tumorigenesis is intraepithelial neoplastic transformation and subsequent intraepithelial proliferation of the transformed cells, which replace or undermine the normal urothelium—the morphologic correlates of this process are dysplasia and, in its more severe form, CIS. Lee and Droller reported that low-grade, mucosally confined papillary tumors develop as a result of cell proliferation, induction of neovascularity, secretion of growth factors that function in an autocrine manner, and production of papillary fronds surrounding a central fibrovascular core. Several growth factors are involved in angiogenesis, and these might have an important role in the regulation of tumor growth. CD10 is known to regulate these factors including platelet-derived endothelial cell growth factor, vascular endothelial growth factor, transforming growth factor β, and endothelin. CD10 is known to hydrolyze endothelin and might have a role in urothelial angiogenesis by modulating endothelin levels. Derangements in CD10-dependent signal transduction pathways that regulate cell growth and apoptosis as a result of abnormal expression of CD10 might have a role in uncontrolled cell proliferation and tumorigenesis.

Alterations at numerous genetic loci have been identified in urothelial tumors. It is interesting that aberrations on the long arm of chromosome 3 (the location of the CD10 gene) are seen in 7% to 24% of IUCs and in 1% to 5% of noninvasive bladder neoplasms, although mutations of the CD10 gene locus have not been documented in urothelial tumors. Based on studies of CD10 in other tissues and tumors, the most likely explanation for the role of CD10 in urothelial neoplasms seems to be via degradation of peptides that are involved in the regulation of tumor growth. CD10 is known to regulate peptide-mediated cell proliferation in many nonhematopoietic tissues. For example, it hydrolyzes BLPs, which are potent mitogens for fibroblasts, alveolar type II pneumocytes, and bronchial epithelial cells and serve as autocrine growth factors in small cell and non–small cell lung carcinomas; these tumors exhibit low or absent CD10 expression. Ganju et al demonstrated an inverse correlation between CD10 expression and proliferative activity in non–small cell lung carcinoma cell lines. Growth of fetal lung, which is BLP-mediated, also is thought to be regulated by CD10.

CD10 is expressed in cells of the genitourinary tract, such as the ductal epithelium of the prostate and epididymis and in their tumors, such as prostatic adenocarcinoma. Studies in prostate carcinomas have shown that CD10 inhibits cell migration via a nonenzymatic protein-to-protein interaction. This explains the correlation of reduced CD10 expression with progression of tumor, particularly in metastatic, androgen-independent prostate cancer; in addition, the tumor is more susceptible to autocrine and paracrine growth factors as a result of the loss of the inhibitory influence of CD10. CD10 expression also is down-regulated with increasing tumor grade in endometrial carcinomas. Thus, CD10 functions as a tumor suppressor because its loss seems to facilitate the development and progression of prostatic, lung, and endometrial tumors.

However, studies of CD10 expression in melanomas have shown increased expression with tumor progression and metastasis. Thus, CD10 might have differing roles in various tumors. In contrast with the patterns of expression of CD10 in lung, prostatic, and endometrial carcinomas, the high-grade group of urothelial neoplasms in our study showed greater intensity and a more diffuse distribution of CD10 staining than the low-grade group. It is possible that the contribution of CD10 to urothelial tumorigenesis is different from its role in prostatic, lung, and endometrial tumors. It also is possible that aberrant expression of wild-type CD10 might occur as a result of mutations in cellular pathways that affect or are affected by CD10. Another possibility is that the increased immunohistochemical expression of CD10 seen in higher grade urothelial tumors reflects accumulation of mutated, nonfunctional CD10 rather than overexpression of normal, functional CD10.

The diagnostic groups showed differences in the distribution of CD10 staining. The high-grade group showed predominantly diffuse staining; a smaller proportion showed deep only staining. The low-grade group showed a mixture of diffuse, superficial only, and deep only staining, whereas the dysplasia group showed an intermediate combination. If the staining within these compartments reflects the predominant sites of gene mutation, the differences in CD10 expression likely correlate with the extent and distribution of genetic damage and are in keeping with the well-known fact that higher grade tumors carry greater amounts of genetic damage than lower grade tumors.

The most significant limitation of our study is the small number of cases tested. Comparisons between individual diagnostic groups, each containing 10 cases, showed certain trends, some of which were statistically significant. It is likely that the significance (or lack thereof) of small differences between these entities will be better elucidated if a larger number of cases is studied. However, when the raw data were analyzed after grouping the cases into low-grade, dysplasia, and high-grade categories (bolstering the numbers within the low-grade and high-grade groups), the differences between them in staining intensity and staining distribution were statistically significant. Further studies with larger numbers of cases might help clarify small differences between CD10 staining profiles of individual diagnostic entities. If such studies were combined with molecular genetic techniques, the issue of whether the
CD10 expressed in urothelial lesions is a result of overexpression of wild-type CD10 or of mutated CD10 also might be resolved, leading to a better understanding of the role of this enigmatic enzyme in urothelial neoplasia.

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References


