

# Application of Immunohistochemistry to the Genitourinary System (Prostate, Urinary Bladder, Testis, and Kidney)

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• **Context.**—The variety of morphologic patterns of different entities of the genitourinary tract can present a diagnostic dilemma for the pathologist. This is especially true in cases of mimics of cancer, a cancer of unknown primary, or poorly differentiated tumors, in which it is hard to assign histogenesis needed to plan the correct therapy for the patient. Immunohistochemistry offers a better capacity than hematoxylin-eosin staining alone to differentiate human tissue types. Also, in the past decades, several techniques had been developed to differentiate between benign and malignant processes with morphologic overlap. By using immunohistochemistry in selected cases, the rate of false-negative and false-positive diagnoses can be reduced, and some patients are afforded the opportunity to get more specific or effective therapy as a result.

Many articles have been written regarding the value of immunohistochemistry (IHC) in lesions of the genitourinary system. In an attempt to compare the results of these studies, the shorthand in Table 1 is used throughout this article (Tables 2 through 5). For purposes of this review, and as shown in Table 1, the results have been divided into 5 categories for each marker.

## PROSTATE

Prostate cancer (PCa) remains the most common malignancy affecting men and the second leading cause of cancer-related death of men in the United States.<sup>1</sup> In 2006, there were 234 460 new cases of PCa, resulting in 27 350 deaths in the United States.<sup>2</sup>

Prostate-specific antigen (PSA) (clone ER-PR8, cytoplasmic staining), discovered approximately 30 years ago, is the most useful among all biomarkers used for the detection of cancer. Serum PSA is useful to distinguish between PCa and benign prostatic hyperplasia, although the test lacks specificity at low-level elevation.<sup>3,4</sup> Historically, the 2 most commonly used primary antibodies in PCa tissue IHC are those to PSA (used almost exclusively in meta-

**Objective.**—For each subgroup of genitourinary system tumors, common diagnostic problems are reviewed, and immunohistochemical markers useful in addressing these problems are discussed, along with expected patterns of immunoreactivity.

**Data Sources.**—The pertinent literature, with focus on immunohistochemical staining of tumors of the genitourinary tract.

**Conclusions.**—The addition of immunohistochemistry to the diagnostic armamentarium for genitourinary pathologic diagnosis has increased the sensitivity and specificity of diagnoses and aided in the selection of optional therapeutic regimens in selected cases.

(*Arch Pathol Lab Med.* 2008;132:432–440)

static deposits) and to high-molecular-weight, basal cell-specific keratin (clone 34 $\beta$ E12, cytoplasmic staining) used for discrimination between benign and malignant proliferations in the prostate. Prostatic secretory cells are immunoreactive for antibodies to broad-spectrum and low-molecular-weight cytokeratins. However, only basal cells express high-molecular-weight cytokeratins.<sup>5</sup> Because uniform absence of a basal cell layer in prostatic acinar proliferations is one important diagnostic feature of invasive carcinoma and basal cells may be inapparent by hematoxylin-eosin stain, basal cell specific immunostains may help to distinguish invasive PCa from benign small acinar proliferations—mimics that retain their basal cell layer (eg, glandular atrophy, postatrophic hyperplasia, atypical adenomatous hyperplasia [adenosis], sclerosing adenosis, and radiation-induced atypia, among others).<sup>6–8</sup> Prostate-specific antigen immunohistochemical analysis is used most often to distinguish PCa from other types of cancer, usually in metastatic sites but occasionally within the prostate. The sensitivity of PSA in high-grade PCa is not as good as the sensitivity in lower-grade PCa, and for that reason, it would be unwise to exclude the possibility of metastatic PCa in a high-grade tumor that is negative with PSA. In this situation, additional markers of prostatic origin are indicated. The most common scenario in which a pathologist uses PSA staining in the prostate would be to distinguish poorly differentiated PCa from urothelial carcinoma (UC) extending into the prostate. Fortunately, PSA immunohistochemical analysis is both highly sensitive and highly specific in this application.<sup>9,10</sup>

More recently, p63 (clone 7JUL, nuclear staining) has

*Immunohistochemistry of Genitourinary System*—Hammerich et al

Accepted for publication May 8, 2007.

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The authors have no relevant financial interest in the products or companies described in this article.

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Category	Sign	Interpretation
Positive	+	Almost all tumors are positive; a negative result would be unusual
High	+/-	Most tumors are positive
Moderate	-/+	Some tumors are positive
Low	-	Most tumors are negative
Negative or rare	0	Almost all tumors are negative; a positive result would be unusual
Unknown	?	Lack of information, results are unknown

Immunostain Class	EpCam	ATM	AMACR	PSA	34βE12	p63	Prostein	NKX3.1
Prostate cancer	+	+	+	+/-	0	0	+	+
Benign prostate	-	-/+	-	+/-	+	+	+	+

\* ATM indicates ataxia-telangiectasia mutated; AMACR, α-methylacyl-CoA racemase; and PSA, prostate-specific antigen.

Immunostain Class	EMA	CK7	p63	TM	CK5/6	EpCam	CD57	PSA	PAP	NKX3.1	Prostein
Urothelial carcinoma	+	+	+/-	+/-	+/-	-/+	-	0	0	0	0
Prostate carcinoma	-	-	0	0	0	+	+	+/-	+	+	+

\* EMA indicates epithelial membrane antigen; CK, cytokeratin; TM, thrombomodulin; PSA, prostate-specific antigen; and PAP, prostate acid phosphatase.

Immunostain Class	PP	OCT4	CD117	AE1/AE3	CAM 5.2	CD30	AFP	hCG	PLAP	HLA-G	Glypican 3
ITGCN/classical seminoma	+	+	+	-	-	-	-	+ STC	+	-	-
Spermatocytic seminoma	?	-	-/+	-	-	0	0	-	-	?	-
YST	0	-	-	+	+	-/+	+	-	-/+	-	+
CC	0	-	-	+	+	-	-	+ STC	+	+ IT	+
EC	-	+	-	+	+	+	-/+	-	+	-	-

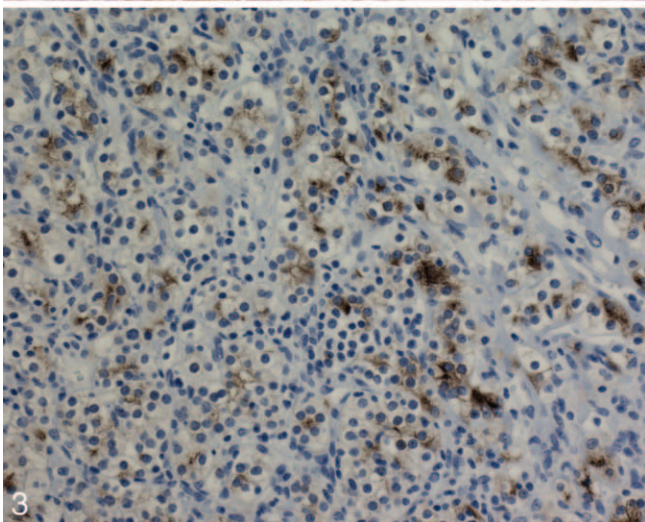
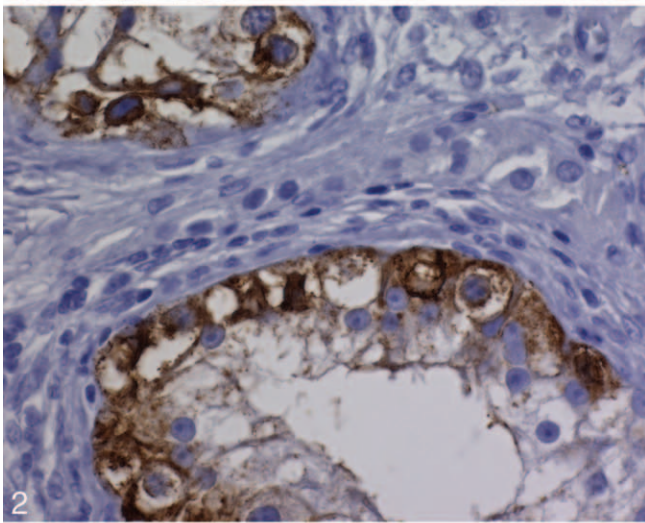
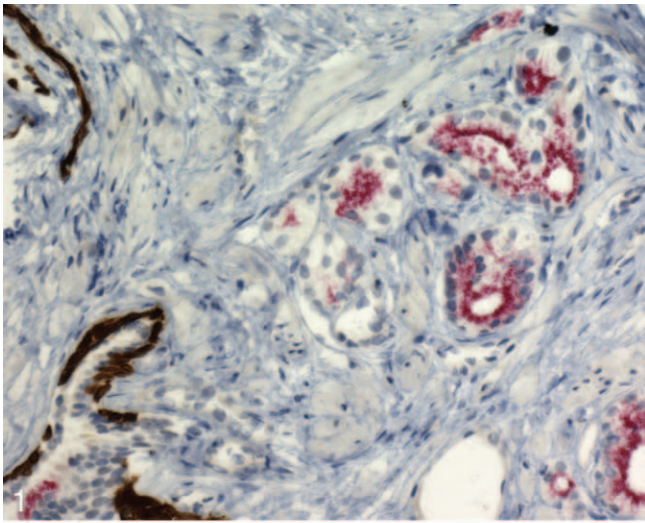
\* PP indicates podoplanin; AE1/AE3, cytokeratin AE1/AE3; AFP, α-fetoprotein; hCG, human chorionic gonadotropin; PLAP, placental-like alkaline phosphatase; ITGCN, intratubular germ cell neoplasia; STC, syncytiotrophoblastic cells; YST, yolk sac tumor; CC, choriocarcinoma; IT, intermediate trophoblastic lesions; and EC, embryonal carcinoma.

Immunostain Class	Parvalbumin	CD10	E-Cadherin	KS-Cadherin	N-Cadherin	CD117	PAX-2	
CCRC	0	+	0	-	+/-	0	+	
RC Chrom	+	-	+	+	0	+	-	
RC Pap	0	-/+	-	-	+	-	-/+	
ROnc	+/-	-	+	+	0	+/-	-	
Immunostain Class	RCC	Aquaporin-1	AMACR	EpCam (MOC-31 and Ber-EP4)	CK7	CD15	Vimentin	CAM 5.2
CCRC	+/-	+/-	-	-	-	+/-	+	+
RC Chrom	0	0	0	+/-	+/-	-	0	+/-
RC Pap	+/-	+/-	+	+/-	+/-	+	+	+/-
ROnc	0	0	-	-	-/+	+	-	+

\* CCRC indicates clear cell renal carcinoma; RC Chrom, renal carcinoma, chromophobe; RC Pap, renal carcinoma, papillary; ROnc, renal oncocytoma; AMACR, α-methylacyl-CoA racemase; and CK7, cytokeratin 7.

been added to the list of markers for basal cells.<sup>11,12</sup> p63 is a nuclear protein encoded by a gene on chromosome 3q27-29 with homology to *p53* (a tumor suppressor gene) and has been shown to regulate growth and development in epithelium of the skin, cervix, breast, and urogenital tract.<sup>13</sup> p63 has the advantage of being a nuclear stain, which is especially useful when combined as a cocktail with α-methylacyl-CoA racemase (AMACR) using a single chromogen.

AMACR (clone P504S, cytoplasmic staining; Figure 1) was identified using expression profiling as preferentially expressed in PCa as compared with normal prostate and confirmed using tissue microarrays.<sup>14-16</sup> Immunohistochemical studies on biopsy material with an antibody directed against AMACR demonstrate that more than 80% of PCa show increased expression over normal prostatic epithelium.<sup>15,17</sup> However, AMACR is not specific for PCa and may be present in nodular hyperplasia (12%), atro-



**Figure 1.** Combo  $\alpha$ -methylacyl-CoA racemase and high-molecular-weight cytokeratin stain of prostate (original magnification  $\times 200$ ).

**Figure 2.** Placental-like alkaline phosphatase in intratubular germ cell neoplasia of the testis (original magnification  $\times 400$ ).

**Figure 3.** Renal cell carcinoma in clear cell carcinoma of the kidney (original magnification  $\times 100$ ).

phic glands, nephrogenic adenoma, and high-grade prostatic intraepithelial neoplasia ( $>90\%$ ).<sup>18</sup> It should be noted that this marker is expressed by a wide variety of non-prostate carcinomas (including UC). Recently, cocktails of high-molecular-weight cytokeratin, p63, and AMACR have been proposed to detect small foci of PCa in prostate biopsies.<sup>19</sup>

Potential future markers include ATM (clone ATML2, nuclear staining). The responsible gene in ataxia-telangiectasia is named ataxia-telangiectasia mutated (*ATM*). The telomere dysfunction and telomerase activation may have an important function in prostate tumorigenesis. Patients with ataxia-telangiectasia tend to develop cancer and show chromosomal instability, abnormalities in genetic recombination, and defective signaling to programmed cell death and several cell cycle checkpoints activated by DNA damage.<sup>20</sup> Because the ataxia-telangiectasia mutated gene product (ATM protein) is involved in maintaining telomere length and integrity, Angèle et al<sup>21</sup> hypothesized that its expression might be altered in prostate tumors. In an experimental study, most patients with a PCa had ATM protein levels higher than those observed in normal tissues. There was also a trend toward higher ATM expression in tumors with a higher Gleason score. These findings support the hypothesis that the presence of the ATM protein might have an important role in the maintenance of the shortened telomeres commonly found in PCa cells.

Went et al<sup>22</sup> showed that EpCam (clone ESA or EGP40, a 40-kd epithelial transmembrane glycoprotein, encoded by the *GA733-2* gene, located on chromosome 4q.1) is highly expressed in PCa. They detected an increased expression of EpCam in hormone-refractory PCa compared with untreated PCa and concluded that EpCam may be a potential target of an advanced therapy. Poczatek et al<sup>23</sup> performed a study on EpCam in benign and malignant tissue of the prostate. Immunostaining was frequently weak and focal in benign epithelium, and in most cases, less than 10% of the basal cells demonstrated immunostaining. However, a strong expression was detected in malignant cells.

Another additional marker that may be worth mentioning is *NKX3.1*. He et al<sup>24</sup> detected this androgen-regulated homeobox NK-class gene, located on chromosome 8p21.2, primarily in the prostate. Several studies have demonstrated that *NKX3.1* is frequently deleted in PCa. These studies described that *NKX3.1* is found in benign prostatic cells but reduced in atrophy and prostatic intraepithelial neoplasia. However, in addition to its restricted expression in prostatic luminal epithelial cells, *NKX3.1* may also be seen in different tissues such as germ cells of the testis and lobular carcinoma of the breast.<sup>25–27</sup> Ali and colleagues<sup>28</sup> described *NKX3.1* at the 2006 United States and Canadian Academy of Pathology meeting as being an excellent nuclear marker of PCa, particularly high-grade PCa, where it significantly outperformed PSA in this regard. In their study, PSA stained only 8 (42%) of 19 of the poorly differentiated PCa cases, whereas 18 (95%) of 19 stained with *NKX3.1*.

Prostein (clone P501S, transmembrane protein) is a prostate-specific marker with a promising therapeutic and diagnostic potential. This marker, located on chromosome 1, was detected exclusively in prostate tissues. Because of this particular expression, prostein could play a crucial role in the diagnosis of an advanced PCa with distant metastases. Xu et al<sup>29</sup> performed one of the first studies to

identify and discuss the diagnostic potential of prostein. Although the expression did not correlate with the tumor Gleason grade, they described the staining as androgen-dependent. Further studies confirmed these results, detecting prostein in both PCa and benign prostatic hyperplasia, so that this marker is clearly a potential target in PCa in future.<sup>29–32</sup>

Therefore, based on our data, EpCam, AMACR, 34 $\beta$ E12, and p63 are useful markers to discriminate between PCa and benign prostatic hyperplasia.

### UROTHELIAL CARCINOMA

Urothelial carcinoma is the second most common malignancy of the urinary tract. In 2006, there were 61 420 new cases of UC, resulting in 13 060 deaths in the United States.<sup>27</sup> A unique distinction of UC is that the recurrence rate is the highest of any carcinoma. Therefore, patient follow-up must be more rigorous than for other cancer surveillance protocols.<sup>33</sup> In most cases of a poorly differentiated carcinoma within the area of the prostate/urinary bladder, morphologic features on the hematoxylin-eosin stain alone are able to distinguish between PCa and UC. However, poorly differentiated carcinomas in this region occasionally have overlapping morphologic features of both neoplasms, making determination of site of origin difficult. Also, unusual morphologic patterns of bladder carcinoma, such as undifferentiated, spindled, lymphoepithelial, or plasmacytoid pattern, may necessitate immunohistochemical confirmation.

There are currently at least 20 distinct cytokeratins that have been identified, and their composition varies among cell types and is also dependent on cellular differentiation.<sup>34</sup> Bassily et al<sup>35</sup> studied the expression of cytokeratin (CK) 7 (clone OV-TL 12/30, cytoplasmic staining) and PSA in poorly differentiated PCa and UC as a way to distinguish between these 2 different tumors. That study showed that PSA was positive in all but one poorly differentiated PCa. A few cases of PCa were focally positive for CK7, but none was positive for both markers. For UC, CK7 was positive marker in the majority of cases, and all UC were PSA negative. Genega et al<sup>36</sup> reached similar results in their immunohistochemical study.

Kaufmann et al<sup>13</sup> and Hameed et al<sup>37</sup> showed in different immunohistochemical studies that the immunoreactivity for p63 in UC was very strong and negative in PCa. The Kaufmann et al study also performed staining for CK5/6 (clone D5/16 B4, cytoplasmic staining), which showed negative staining in PCa. These results were confirmed by 2 additional studies, Goldstein<sup>38</sup> and Skinnider et al.<sup>39</sup> In all these studies, the immunoreactivity for CK5/6 for UC varied but was significantly higher than in PCa. Because of a lower expression of CK5/6 in UC (except for foci of squamous differentiation), Kaufmann et al suggested that the combination of both markers, CK5/6 and p63, could be necessary to use a highly specific marker combination in poorly differentiated carcinomas to distinguish PCa from UC.

Mhaweck et al<sup>40</sup> evaluated the use of a panel of antibodies to distinguish the poorly differentiated forms of a UC and PCa. Thrombomodulin (clone DAKO-THR 1009, endothelial cell transmembrane staining), a surface glycoprotein, and prostate acid phosphatase (clone PASE/4LJ, cytoplasmic staining) were the primary focus in this retrospective study. The results showed a moderate reactivity for thrombomodulin in UC, whereas PCa was negative.

Prostate acid phosphatase was highly expressed in PCa and negative in UC. In 2 independent studies of Ordonez<sup>41</sup> and Parker et al,<sup>42</sup> thrombomodulin expression was present in most UC and was negative in PCa.

McKenney and Amin<sup>43</sup> studied the use of CD57 (clone Leu-7, located in cell membrane of lymphoid cells in germinal centers) in PCa and in UC. CD57 is a membrane antigen seen in approximately 20% of peripheral blood mononuclear leukocytes, a proportion of which have “natural killer” activity. McKenney and Amin showed CD57 expression to be a supportive marker of prostate carcinoma, whereas only a minority of UC stained positive. Similar results were reported by Kaufmann et al.<sup>44</sup>

McKenney and Amin<sup>43</sup> also analyzed the epithelial membrane antigen (clone 214D4, cytoplasmic staining). Epithelial membrane antigen is one of several human milk fat globule proteins. These compose part of the plasma-membrane of epithelial cells in areas of the cell membrane overlying tight junctions.<sup>45</sup> These studies demonstrated a significantly higher immunoreactivity for epithelial membrane antigen in UC versus PCa.<sup>46–48</sup>

The expression of EpCam occurs in normal epithelium of different organs and has been described in carcinomas of various sites.<sup>49</sup> Because of its strong expression in carcinomas of various sites, EpCam has gained interest as a potential therapeutic target and is being evaluated in clinical trials. In analyzing this marker, Went et al<sup>22</sup> showed an increased expression in PCa with a high fraction of strongly positive tumors. On the other hand, in UC EpCam expression was very weak. Therefore, they concluded that EpCam may be a good marker to distinguish between PCa and UC. (Please see the Note at the end of the article.)

Prostein and NKX3.1 are both described previously. Because prostein is highly restricted to prostate tissue, it is a useful marker to distinguish between poorly differentiated PCa and UC.<sup>28,29</sup> A few studies have demonstrated the specificity of NKX3.1 to primary and metastatic prostate tumors, and because it is highly expressed in PCa, it is also a useful marker to distinguish poorly differentiated PCa from high-grade UC. In UC, the detection of this marker was negative.<sup>50,51</sup>

Therefore, based on these data, epithelial membrane antigen, PSA, CK7, EpCam, CD57, prostate acid phosphatase, NKX3.1, and prostein are the most useful markers to distinguish UC from PCa.

### TESTICULAR NEOPLASMS

Testicular neoplasms are uncommon, comprising about 1% of malignant neoplasms in males.<sup>52</sup> In 2006, there were 8250 new cases of cancer of the testis, resulting in 370 deaths in the United States.<sup>2</sup> The majority (~95%) of testicular neoplasms are germ cell tumors (GCTs) (eg, seminoma, embryonal carcinoma, yolk sac tumor, teratoma, choriocarcinoma, and mixed variants of these). Approximately 40% are pure seminoma, 20% are pure embryonal carcinoma, and 40% are mixed tumors that contain 2 or more components. Advances in IHC and other molecular testing methods have supplemented routine hematoxylin-eosin staining in differential diagnosis, especially in metastatic carcinoma of unknown primary site. In tumors that are cytologically anaplastic and have no architectural differentiation (ie, solid growth pattern), IHC may be required to pinpoint the exact diagnosis. Intratubular germ cell neoplasia (ITGCN), being a preinvasive GCT, may be

diagnostically challenging. This is especially true in biopsy specimens of the testis contralateral to an invasive GCT, a situation in which the presence of ITGCN requires additional therapeutic measures.

$\alpha$ -Fetoprotein (AFP) (cytoplasmic staining), placental-like alkaline phosphatase (cytoplasmic staining; Figure 2), and human chorionic gonadotropin (hCG) (cytoplasmic staining) have had a special role in immunohistochemical analysis of GCTs of the testis and are well established. Several studies demonstrated that yolk sac elements express AFP, whereas hCG stains positive in syncytiotrophoblastic giant cells.<sup>53-56</sup> Placental-like alkaline phosphatase is expressed in seminoma, embryonal carcinoma, and choriocarcinoma, with patchy staining in yolk sac tumor.<sup>53-56</sup> Placental-like alkaline phosphatase is also a useful marker in ITGCN, whereas hCG and AFP are negative<sup>57-62</sup> (Figure 2). In spermatocytic seminoma, placental-like alkaline phosphatase and AFP are both negative.<sup>63,64</sup> However, it should be kept in mind that placental-like alkaline phosphatase is also seen in a significant number of non-germ cell carcinomas.<sup>53-56</sup>

Podoplanin (clone M2A, located in cell membrane, diagnostically equivalent to D2-40<sup>65</sup>), a small 38-kd mucin-type transmembrane glycoprotein, has been implicated in tumor progression in a variety of human cancers. The expression of podoplanin is upregulated in squamous cell carcinoma of the oral cavity, lung, and skin; granulosa cell tumors; and mesothelioma.<sup>66</sup> In a study by Bailey et al,<sup>67</sup> it was shown that staining for M2A is virtually 100% in seminoma. In sharp contrast, in nonseminomatous germ cell tumor (NSGCT), the reactivity for M2A is negative. Two studies, Sonne et al<sup>68</sup> and Biermann et al,<sup>58</sup> demonstrated positive expression for podoplanin in ITGCN. To our knowledge, there is nothing published about the immunohistochemical staining of HLA-G in spermatocytic seminoma.

OCT4 (clone POU5F1, nuclear staining), a transcription factor that maps to the human chromosome 6p21.3, is involved in regulation of pluripotency during normal development. It is detectable in embryonic stem cells and germ cells and is virtually 100% sensitive and specific for seminoma and embryonal carcinoma, including ITGCN.<sup>69,70</sup> Looijenga et al<sup>71</sup> analyzed the presence of OCT4 in GCT and other tumor types using IHC. The immunostaining of this marker was consistently and only positive in seminoma, germinoma, dysgerminoma, embryonal carcinoma, and ITGCN. These results are also in line with the findings of Ulbright and Young<sup>72</sup> who identified a positive staining of OCT4 in all classical seminoma, in contrast to a negative expression in spermatocytic seminoma. Jones et al undertook immunohistochemical staining of OCT4 in testicular neoplasms. In almost all cases of ITGCN the expression was very strong, confirmed by Biermann's subsequent study.<sup>57,58</sup>

Cytokeratin is also helpful in distinguishing seminoma from a solid type of embryonal carcinoma. In Ulbright's study, seminoma and spermatocytic seminoma were negative with murine monoclonal antikeratin antibodies, cytokeratin AE1/AE3 (cytoplasmic staining).<sup>72</sup> Several other studies have shown this antikeratin cocktail to have weak expression in seminoma but strongly positive staining in embryonal carcinoma.<sup>73,74</sup> The identification of the perinuclear dotlike pattern of reactivity with cytokeratin in seminoma may be the first clue in an unusual site (eg, lymph node of neck) that one is dealing with metastatic

seminoma. Studies analyzing the expression of AE1/AE3 in ITGCN are rare. Bruce et al<sup>75</sup> demonstrated a negative staining for this marker in a small series of ITGCN.

The CD30 antigen (clone KI-1, located in cell membrane) has been used primarily as a diagnostic marker for Hodgkin disease. The solely described nonhematopoietic malignancy with a strong expression for CD30 is embryonal carcinoma. In other types of testicular GCT an equivalent positive staining for CD30 has not been reported.<sup>76,77</sup> Hittmair et al<sup>78</sup> detected a specific positive immunostaining for CD30 in seminoma only as a few foci in contrast to strong reactivity in embryonal carcinoma. In this study, the staining for CD30 in spermatocytic seminoma was negative. Further studies have confirmed the expression of CD30 in embryonal carcinoma and that other types of GCT are essentially negative.<sup>73,74,79,80</sup> In a small study, Zamecnik and Sultani<sup>81</sup> demonstrated a negative expression for CD30 in ITGCN.

CAM 5.2, a low-molecular-weight cytokeratin with cytoplasmic and membrane staining, was analyzed in several immunohistochemical studies, and strong positivity was shown in NSGCT. In seminoma and spermatocytic seminoma, the expression was only focal and very weak. The studies' authors concluded that this marker could be very helpful in distinguishing seminoma from NSGCT.<sup>46,53,73</sup> To our knowledge, there are no data published analyzing the staining of CAM 5.2 in ITGCN.

CD117 (clone C-KIT; CMA-767; 2E4; A4502) is a transmembrane tyrosine kinase receptor protein encoded by the proto-oncogene *c-kit* that maps to chromosome 4 (4q11-12). The *c-kit* proto-oncogene product and its ligand stem cell factor play an important role in hematopoiesis, spermatogenesis, and melanogenesis. In different studies, several authors detected CD117 as a characteristic marker for seminoma to separate this tumor from NSGCT. They described a strong reactivity in seminoma, with NSGCT showing only a very weak staining. Because of the positive staining for CD117 and the negative staining of CD30 in seminoma, and the opposite staining in NSGCT, the authors concluded that the combination of both markers could represent a valuable tool for distinguishing between these 2 entities.<sup>82-85</sup> Reuter<sup>86</sup> confirmed the finding by Dieckmann and Skakkebaek<sup>87</sup> that CD117 is overexpressed in ITGCN. In an immunohistochemical study, Kraggerud et al<sup>88</sup> demonstrated a strong positive staining for CD117 in all analyzed seminoma. Only a few (41%) spermatocytic seminomas stained positive for this marker.

Another useful marker is the HLA-G antibody (clone 4H84), an excellent marker of intermediate trophoblastic cells. This antibody interacts with the nonclassical major histocompatibility complex class I antigen, located on the cell surface.<sup>89</sup> Kurman et al<sup>91-92</sup> demonstrated in several studies the specificity of this marker for trophoblastic tumors such as choriocarcinomas. In especially difficult situations concerning a metastatic high-grade cancer of unknown primary site, this marker is a promising and useful tool to detect trophoblastic differentiation. Inhibin and hCG are useful as well in the immunohistochemical demonstration of trophoblastic differentiation.<sup>92-95</sup> To our knowledge, there is nothing published about the immunohistochemical staining of HLA-G in spermatocytic seminoma.

In a recent study, Zynger et al<sup>96</sup> analyzed glypican 3 (GCP3) as a useful marker for subtypes of germ cell neoplasms. They demonstrated the GCP3 expression in 100%

of yolk sac tumor and 100% of choriocarcinoma components compared with only 8% of embryonal carcinoma and 38% of teratomas with immature elements. Seminoma and ITGCN were consistently negative. This marker could be promising in identifying nonseminomatous components and distinguishing yolk sac tumors from other germ cell neoplasms.

To discriminate between NSGCT and classical seminoma, podoplanin, OCT4, CD117, AE1/AE3, CAM 5.2, CD30, and AFP are all useful markers. To distinguish among the subtypes of NSGCT, OCT4, CD30, AFP, and GCP3 may be helpful. Syncytiotrophoblastic cells are best detected with hCG.

## RENAL NEOPLASMS

Renal cell carcinoma is the most lethal of all urologic tumors with approximately one third of afflicted patients dying of the condition. In 2006, there were 38 890 new cases of kidney cancer, resulting in 12 840 deaths in the United States.<sup>2</sup> The most common subtype of kidney cancer is clear cell renal carcinoma (CCRC), accounting for about 85% of the total and 2% of all cancer deaths.<sup>97,98</sup> During the last 3 decades, several additional subtypes of epithelial renal cancer have been described that had been previously included under the general category of renal cell carcinoma. Because there is occasional morphologic overlap among the different subtypes, IHC may be necessary to arrive at a conclusive diagnosis.

Several diagnostic studies have been performed concerning CD10, a 100-kd type II cell surface metalloendopeptidase (clone 56C6; NCL-270). In most of these studies, CD10 was strongly positive for CCRC. In 1 representative study, Mazal et al<sup>99</sup> analyzed the immunostaining of CD10 in a large group of patients and found that CD10 expression was positive in approximately 85% of CCRC, 23% of papillary renal cell carcinoma (RC Pap), 4% of chromophobe renal cell carcinoma (RC Chrom), and 3% of renal oncocytoma (ROnc). Similar results were obtained in studies by Kim and Kim<sup>100</sup> and Avery et al.<sup>101</sup> These studies confirmed that most CD10-positive renal neoplasms are CCRC and RC Pap. It should be noted that CD10 is also positive in a wide variety of non-renal cell carcinomas.

Cytokeratin reactivity has also been studied. Mazal et al<sup>99</sup> demonstrated CK7 positivity in 39% of all renal carcinomas and in 7% of ROnc. CCRC stained positive in 8%, in contrast to RC Pap (77%) and RC Chrom (88%), confirming the results of Kim and Kim<sup>100</sup> and Delahunt and Eble.<sup>102</sup> This particular CK7 (clone KS7.18; LDS-68; OVTL 12/30) was analyzed in several additional studies. Almost all RC Pap and RC Chrom strongly expressed CK7, whereas CCRC was negative. In most of these studies, CK7 staining of ROnc was weak or focal.<sup>39,99,100,102</sup>

Low-molecular-weight cytokeratin (clone CAM 5.2) is also a useful additional marker. Several studies demonstrated a consistently moderate to strong expression of CAM 5.2 in CCRC.<sup>103–105</sup> However, Stopyra et al<sup>106</sup> detected a strongly positive staining for CAM 5.2 in ROnc.

Aquaporin-1, an antibody against water channel proteins of the plasma membrane, was studied by Mazal et al<sup>99</sup> who demonstrated that this marker could be a promising tool to distinguish among CCRC (78%), RC Pap (73%), RC Chrom (0%), and ROnc (0%).

PAX-2, a nuclear transcription factor instrumental in renal development, is another tool in the differential diagnosis of renal neoplasia. Mazal et al<sup>99</sup> demonstrated a sen-

sitivity of 88% in CCRC but a considerably lower expression in RC Pap. Daniel et al<sup>107</sup> performed one of the first studies using PAX-2 in diagnosis of renal neoplasia. In that study, PAX-2 was strongly expressed in 83% of RC Pap. In CCRC the expression was higher (93%) but varied in intensity. Importantly, negative staining was noted in ROnc and RC Chrom. However, in a very large study, Mazal et al<sup>99</sup> found similar result of PAX-2 staining in CCRC (88%) but demonstrated a patchy expression in ROnc (14%), RC Pap (18%), and RC Chrom (13%).

Vimentin (clone 3B4; RPN1102; V10; V9) is a member of the intermediate filament family of proteins and is an important structural feature of eukaryotic mesenchymal cells. Vimentin, along with microtubules and actin microfilaments, make up the cytoskeleton. Skinnider et al<sup>39</sup> demonstrated that this marker was commonly expressed in 87% of CCRC and 100% in RC Pap, whereas vimentin was rarely expressed in RC Chrom (7%) and ROnc (10%). These expression patterns were confirmed in several studies. Therefore, vimentin is useful to distinguish between CCRC or RC Pap versus RC Chrom or ROnc.<sup>39,108,109</sup>

Cadherins are calcium-dependent cell adhesion molecules. They play an important role in embryonic morphogenesis and the formation of solid tissues. Kidney tubules express these molecules. There are several known subtypes of cadherin, and 3 of those were analyzed in different studies regarding renal neoplasms. Tani et al<sup>110</sup> demonstrated that ROnc stained positive for E-cadherin (clone HECD-1; ECCD-2; ECH-6; 4A2C7 ao, cytoplasmic and membrane staining) but negative for N-cadherin (clone 13A9; 3B9; GC-4, membrane staining). RC Pap stained negative for both E-cadherin and N-cadherin. Taki et al<sup>108</sup> confirmed these results with 100% of RC Chrom and ROnc positive for E-cadherin but negative for N-cadherin, whereas 100% of CCRC were negative for E-cadherin and 58% positive for N-cadherin. Ordonez<sup>111</sup> detected N-cadherin in 100% of CCRC and RC Pap. In a large cohort, Shen et al<sup>112</sup> analyzed the expression of kidney-specific cadherin (membrane staining). In almost all RC Chrom (100%) and ROnc (95%), kidney-specific cadherin was expressed in contrast to only 14% of CCRC and 13% of RC Pap. They concluded that kidney-specific cadherin is a promising and sensitive novel marker to distinguish RC Chrom and ROnc from other epithelial neoplasm of the kidney.

Another useful marker is RCC, a monoclonal antibody against a normal human proximal tubular brush border antigen with cytoplasmic staining (Figure 3). In a study by Shen et al,<sup>112</sup> 86% of CCRC and 97% of RC Pap stained positively, whereas only of 15% of RC Chrom and 0% of ROnc were positive. Other studies have confirmed these results.<sup>101,113,114</sup> Avery et al<sup>101</sup> concluded that a high percentage of clear cell carcinomas express cell surface RCC, therefore making RCC a promising discriminator between metastatic CCRC and clear cell carcinomas arising in other sites.

Numerous studies analyzed the role of CD117 in renal carcinoma. In a large group of patients, Pan et al<sup>115</sup> demonstrated that CCRC and RC Pap stained negatively, whereas most ROnc (71%) and RC Chrom (83%) were positive for CD117. Lin et al<sup>116</sup> showed positive staining in 100% of RC Chrom and RC Pap and 0% of CCRC. Other studies have confirmed these results, affirming that CD117 is a useful marker in the differential diagnosis of subtypes of renal carcinoma.<sup>113,117</sup>

Several studies analyzed the staining pattern of CD15 (clone 3C4; 3CD1; C3D1; CBD1, surface membrane and paranuclear staining) by IHC in CCRC.<sup>108,111,118,119</sup> These studies showed that RC Pap is positive for CD15, with CCRC showing a moderate positive staining and RC Chrom having only weak expression. Of note, all ROnc stained positive for CD15 (100%), but the total number of such patients was relatively small.

A few other novel markers have been analyzed in small studies. Went et al<sup>120</sup> showed that EpCam (clone VU-1D9), an epithelial adhesion molecule expressed in a broad range of carcinomas, could be a helpful tool to differentiate RC Chrom from ROnc. Other EpCam antibodies, such as MOC-31 and Ber-EP4, are also useful for this purpose. Went et al demonstrated that 87% of RC Chrom and 11% of ROnc expressed EpCam. CCRC had a moderate staining (28%), and in RC Pap the staining was very patchy.<sup>22,120</sup> Martignoni et al<sup>121,122</sup> reported in their pathologic studies that all RC Chrom (100%), including metastases, showed a strong immunoreactivity for parvalbumin (clone PA-235), a 12-kd calcium-binding protein, whereas CCRC and RC Pap were negative (0%). However, ROnc showed moderate expression (70%). They concluded that this marker is useful in distinguishing primary and metastatic RC Chrom from RC Pap and CCRC from ROnc. Others have not found this antibody useful (R. Miller, written communication, 2006). AMACR could also have a potential use in the differential diagnosis of renal neoplasms. Tretiakova et al<sup>123</sup> demonstrated a strong expression in CR Pap (100%) and a weak or negative expression in CCRC (25%), ROnc (15%), and CR Chrom (0%).

In summary, in distinguishing CCRC from RC Pap, CD10, CK7, PAX-2, AMACR, and CAM 5.2 are the most useful markers. Parvalbumin, aquaporin-1, PAX-2, CK7, CD10, all types of cadherins, CD117, AMACR, RCC, and vimentin may be included in the differential analysis to discriminate between CCRC and RC Chrom. To discriminate between RC Chrom and ROnc, EpCam, CK7, CD15, and CAM 5.2 are useful.

## CONCLUSION

Hematoxylin-eosin staining is sufficient for the diagnosis of the majority of genitourinary tumors. However, in diagnostically challenging cases, there is currently no special technique that has influenced the way in which pathology is practiced as profoundly as IHC. The addition of IHC to the diagnostic armamentarium for genitourinary pathologic diagnosis has increased the sensitivity and specificity of diagnoses and aided in tailoring the patient to a more appropriate therapeutic regimen in selected cases.

**Note.**—In a few studies, the expression of EpCam was reported to be positive predominantly in high-grade and advanced-stage UCs.<sup>124–126</sup>

We are indebted to Rodney Miller, MD, for his review of this manuscript and many helpful suggestions.

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