13 Diagnostic Values of Immunohistochemistry in Bladder Cancer

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General Considerations

Immunohistochemistry (IHC) has been used as a valuable tool in our surgical pathology practice for more than 50 years. Since then more and more biomarkers have been developed, introduced, and applied in our daily practice. This tool has revolutionized the field of surgical pathology and offered relatively objective parameters and evidence-based support for our diagnoses, prognoses, and potentially therapeutic correlation.

There are always new biomarkers published in the literature; and they typically generate much excitement with an initial report of high specificity. However, as more studies are performed with more sensitive detection systems, its specificity usually decreases. In our practice, we do not introduce all the new antibodies that become popular, unless they can offer new information that is not available by current well-established ones in the lab.

With recent advances in molecular studies of urinary bladder cancers, many new diagnostic markers have been identified and reported in the literature. Like any other organ, application of IHC in bladder pathology should follow the same general considerations. In this chapter, we will discuss the utilities of IHC with focus on the practical pearls and pitfalls in some commonly seen diagnostic challenges instead of reviewing all the established and new markers.

When Do We Need to Request IHC?

There is no clear-cut guideline regarding when IHC should be used. Each pathologist may have a different threshold, because of a different level of confidence secondary to various backgrounds and experiences. When and how to use this tool is more like a combination of science and art. Personally, we request immunostains when we feel the features are not typical for a certain entity, and different interpretations may be rendered if this case is shown to different pathologists. Another important parameter for us is the clinical implications, as we want the patients to be managed with solid evidence.

In most of the cases, the routine hematoxylin-eosin (H&E) stain demonstrates typical features that make us confident about the diagnosis of bladder cancer, and immunostains are not needed. However, for a subset of cases, the histology is not typical and presents some overlapping features between two or even more possible

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diagnoses. At this time, ancillary tests may offer additional evidence for an accurate diagnosis.

We frequently hear "H&E stain trumps the immunostains." Our personal view is that it is usually true; however, it is individually case based. Most of the time, the immunostains should confirm what we think based on the H&E sections. Only rarely should it be "I am glad I ordered the immunostains," a sign that the immunostains offered some additional information that we did not feel confident or were not in favor of it with only on the H&E sections. Even more rarely, we wish we never requested those immunostains, which further muddied the water, and we do not know what to do with all the immunostains in a certain case. A too generous and casual use of IHC without justification could generate more confusion to us rather than providing help, because the different expressions of biomarkers may point us in different directions.

What Panel Do We Need to Pick?

IHC is considered an ancillary test which can support, but not replace, careful morphological evaluation. The practical approach is to form a short list of differential diagnoses based on the histology of the lesion. Look into the clinical setting and understand the clinical impacts of our possible diagnoses. A panel of biomarkers that are complimentary to each other among the differential diagnoses should be used to increase the diagnostic accuracy.

To choose the proper panel of immunostains, we need a strong basic, constant study, innovative and creative thinking, and enjoyment of proper selection (BEST approach). Also, 3C practice (consultation, communication, and collaboration) is commonly required.

Because of the biological nature of a tumor and technical issues associated with the IHC procedures, none of the IHC markers is 100% specific and 100% sensitive in any lesions, including bladder lesions. We must use these markers with justification and caution. Usually we should not rely on one single immunostain, to avoid a false-positive or false-negative result.

How to Interpret the Results?

We need to be aware of the approximate sensitivity and specificity for each biomarker that is used in the panel. Sensitivity and specificity for any given antibody is relative, and so far we do not have absolutely specific and sensitive antibodies. There are at least four issues need to be considered for immunostaining interpretation:

1. What cellular compartment is stained: nuclear, cytoplasmic, membranous, or both nuclear and cytoplasmic.
2. When the marker stains cytoplasm, check whether it is membranous, granular cytoplasmic, or fibrillar cytoplasmic.
3. Check whether the tumor cells stain or entrapped normal cell stain.
4. Check the degree of staining: strong or weak or diffuse or focal.

The signal location is fundamental in our immunostain interpretation. There are three possible signal locations, namely, nuclear, membranous, and cytoplasmic. Also possible is the combination of different locations. It is extremely important to remember the expression pattern for each marker. GATA 3, PAX2/8, and p63 are nuclear patterns. Cytokeratin and racemase are both cytoplasmic markers, but cytokeratin should be fibrillar stain and racemase granular stain. Uroplakin is membranous expression. Some markers may present combined staining patterns, such as S-100, which manifests both cytoplasmic and nuclear immunoreactivity.

How to define a positive stain is essential. The lesional area must be present and recognized, and the targeted cells have to be positive or negative; not all brown stain is necessarily positive. Ideally, non-lesional normal or benign tissue is present to control the immunostained slides. The cells of interest in the immunostained slides may be hard to appreciate when evaluated by hematoxylin counter stain only. Therefore, H&E sections should be reviewed and compared with the immunostains to make sure that we are interpreting the lesional tissue in the right areas.

The Economics of the IHC

Economy should not be the primary consideration when we handle a difficult case. Cost-effectiveness can be achieved by careful planning. Sometimes a phone call with related physicians can save much time and the number of immunostains. Clinical history and imaging results will help shorten the list of our differential diagnosis; subsequently we can use fewer immunostains. Under the new economic-medical climate, efficient utility is extremely important to sustain the lab and practice.

Practical Approach in Specific Diagnostic Dilemmas

There are many diagnostic dilemmas with overlapping histologic features, and yet they are clinically relevant. Now we use some frequently encountered diagnostic dilemmas in bladder pathology to discuss the histologic features, complimentary immunostain panel, signal location, interpretation skills, and clinical implications.

Flat Urothelial Lesions

This group of lesions includes urothelial carcinoma (UC) in situ, dysplasia, proliferation of uncertain malignant potential, and reactive atypia [1]. To separate them from each other is extremely important and not always easy. Several markers are valuable in this setting (Fig. 13.1 and Table 13.1).

CK20 is a marker often applied in the evaluation of flat lesions of bladder. The key is how to interpret its expression pattern. In normal and benign reactive urothelium, CK20 is restricted to the surface umbrella cells. In contrast, CK20 is positive in the full urothelial thickness of dysplastic urothelium or in situ carcinoma (CIS) [2–4]. CK20 cannot separate dysplasia from UC in situ, which relies on histologic evaluation.

P53 is often used in this setting. In normal and reactive urothelium, p53 is usually of scattered and weak nuclear expression. In dysplastic urothelium and CIS, p53 is often diffusely and strongly expressed [2, 3].

Ki-67, a marker for proliferation index, is usually high with whole layer distribution while low and limited to basal and suprabasal layers of normal urothelium [3, 4].

CD44 is also reported to be useful, with an inversed expression pattern with CK20. Namely, it is positive with a membranous pattern in the benign basal and suprabasal cell layers [2]. This membranous expression of CD44 is lost in CIS, particularly the pagetoid type of CIS. It is not hard to understand why some pathologists like to use the combination of these two complimentary markers together to evaluate flat urothelial lesions.

AMACR is another maker that can be used to differentiate reactive atypia from CIS, which is usually positive in CIS while negative in benign reactive urothelium. Comparing with CK20, AMACR was less sensitive and more specific with the same caveat of less staining intensity [5, 6].

Cocktails containing two or three antibodies have been applied on the same slide, offering different color detection and complimentary expression patterns [7, 8].

It should be kept in mind that the IHC in the differential diagnosis of flat urothelial lesion is limited, such as CK20 can be totally lost in CIS and only ~50% CIS has P53 mutation and shows abnormal P53 expression by IHC. Ki-67 labeling can be increased in reactive urothelium, such as inflamed urothelium.

Histologic Variants of Infiltrating UC

This is a very important topic; Chap. 6, "Morphological Variants of Invasive Urothelial Carcinoma," is completely dedicated to the details, including histologic features, immunoprofile, and clinical significance.

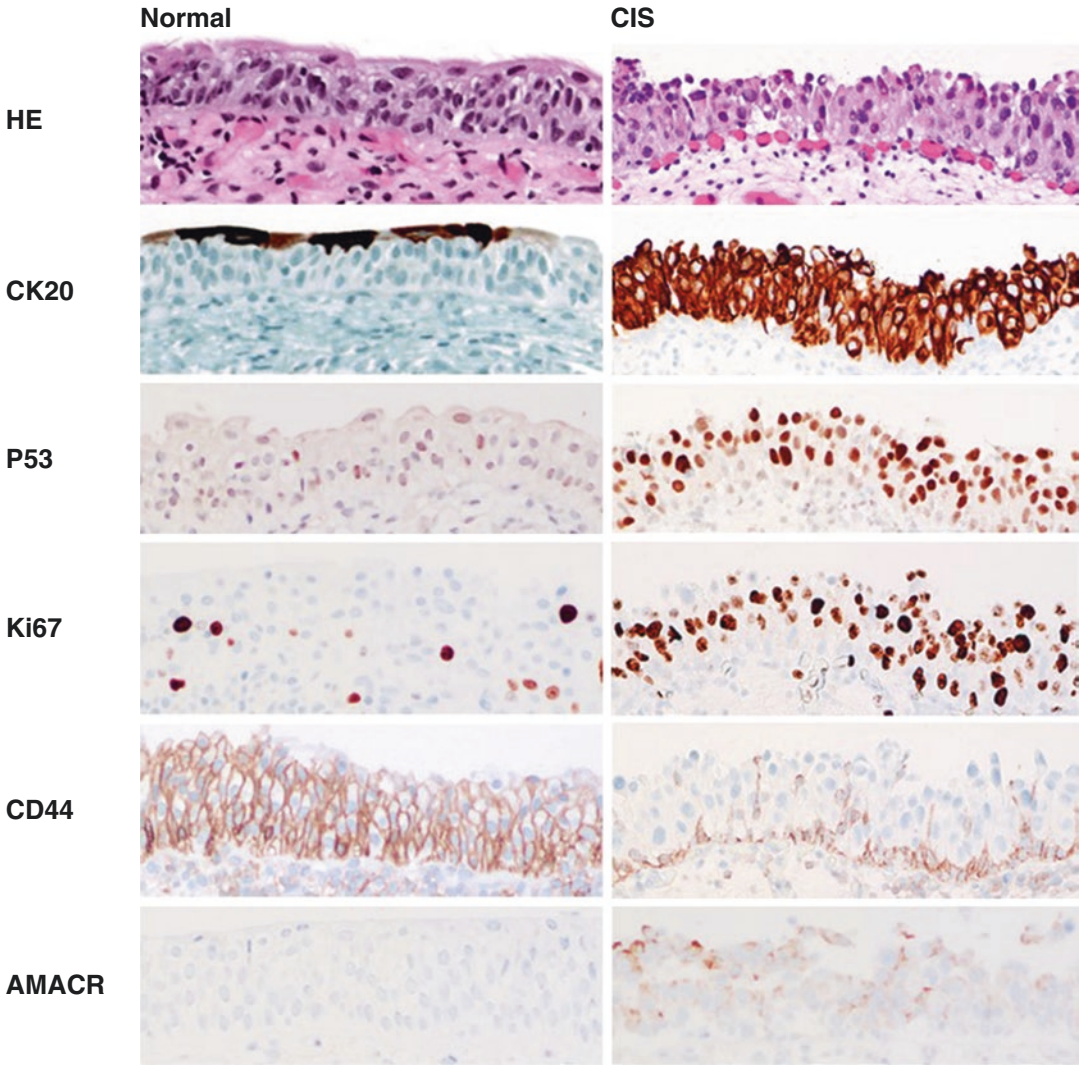

Fig. 13.1 IHC panel for differentiation of benign urothelium from urothelial carcinoma in situ

Table 13.1 IHC panel for differentiation of reactive urothelium from urothelial carcinoma in situ

	Normal	Reactive	Carcinoma in situ
CK20	+ (limited to surface umbrella layer)	–	+ (transurothelial), ~70%
P53	+ (scattered, weak)	+ (scattered, weak)	+ (strong and diffuse)
CD44	+ (limited to the basal)	+ (transurothelial)	– (or limited to basal +)
Ki-67	Low	Moderate to high	High
AMACR	+ (transurothelial), ~70%	–	–

Assess the Depth of an Invasive Urothelial Carcinoma on the Biopsy and Transurethral Resection Specimens

In bladder biopsy or transurethral resection of prostate (TURP) specimens, the depth of invasion of infiltrating UC is critical for the clinician to design the most appropriate subsequent therapeutic approach, cystectomy, or more conservative procedure. Most of the time, we can handle these cases with confidence based on H&E sections alone; however, in difficult cases IHC may offer additional evidence to support our H&E impression.

Smoothelin is reported to be specifically immunoreactive with the contractile muscle bundles, which are muscularis propria (MP); therefore, it is used to distinguish muscularis propria from muscularis mucosae (MM) [9–11]. A diffuse and strong staining pattern is specific and can be considered as MP; on the other hand, a weak and blush pattern is usually considered as MM (Fig. 13.2). However, MP can be weakly stained; therefore, strong and diffuse stain is only useful in this situation. Occasionally smoothelin immunostain can be difficult to interpret; and pitfalls should be kept in mind [12]. If this is the case, smoothelin is not reliable.

Careful lab validation with different conditions/protocols and personal experience are very important. As with any other markers, smoothelin will not solve all the problematic cases. Occasionally, we are not confident whether the muscle bundles represent hyperplastic MM or true MP. It is critical that we communicate with the urologist and comment that we are not sure based on the pathological features. The urologist can either proceed with the imaging findings or perform a very close follow-up and/or re-biopsy of a deeper portion to obtain a more straightforward diagnosis.

Most clinicians including urologists, medical oncologists, and radiation oncologists use muscle involving urothelial carcinoma as interchangeable with urothelial carcinoma involving the MP. So, it is not recommended to use invasive urothelial carcinoma involving muscle bundles in our pathology report; we need to clearly specify it is MM or MP or not sure for MM or MP. Potential misunderstanding should be avoided in this setting, because the clinical implications are dramatic. Patient may undergo an unnecessary cystectomy based on a vague terminology.

Establish the Urothelial Lineage and Rule Out Metastasis

GATA3

GATA3 was described a few years ago, which was considered most specific for urothelial

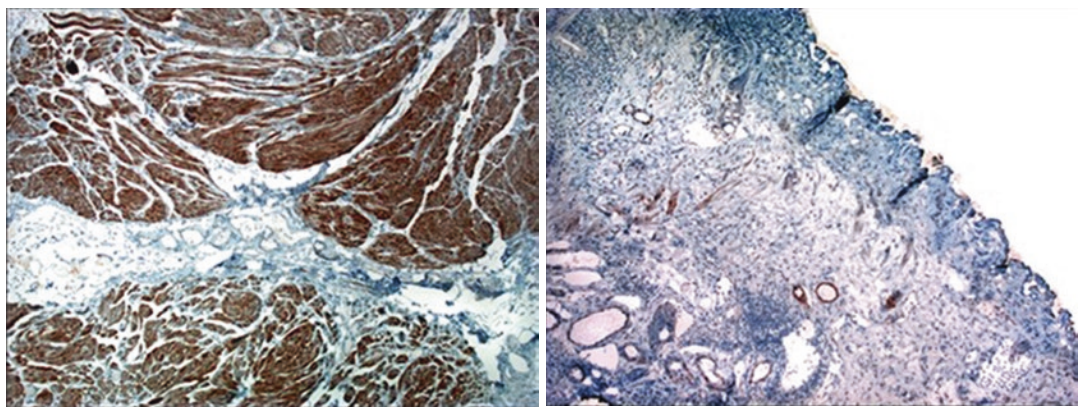

Fig. 13.2 Smoothelin to differentiate muscularis propria from muscularis mucosae

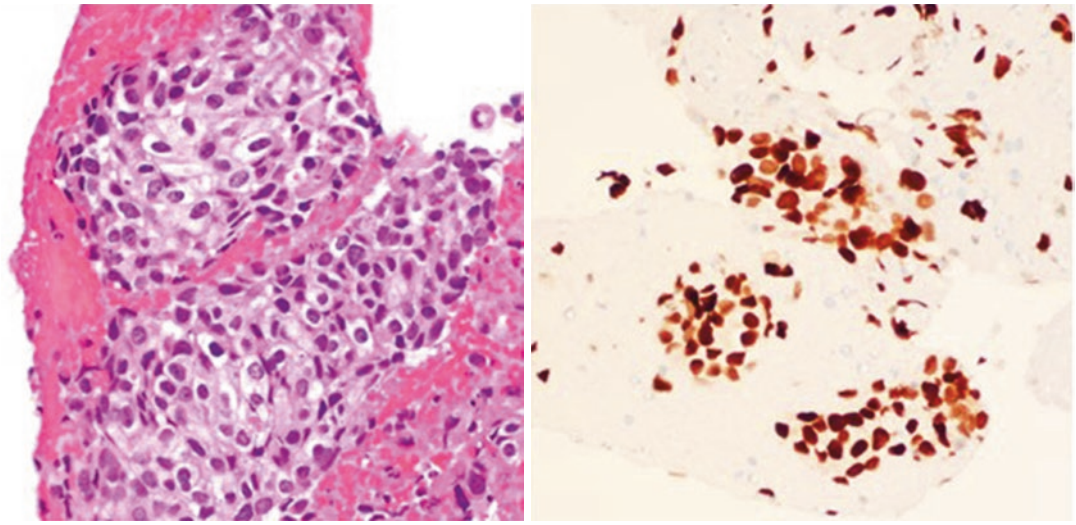

Fig. 13.3 A small biopsy of urothelial carcinoma with GATA3 reactivity

differentiation [13]. GATA3 has higher sensitivity than p63 and CK20 on high-grade urothelial carcinoma, as high-grade (HG) UC usually partially or even totally lose p63 or CK20, while most of these cases retain the expression of GATA3 (14 and Fig. 13.3). Like most immunohistochemical markers, its sensitivity increased, and its specificity declined significantly in the subsequent literature. However, it is still a valuable nuclear marker, particularly when used along with a well-designed panel based on the histology. We should be aware that GATA3 is positive in most of breast carcinomas and many other tumor types [15]. Clinical history and additional urothelial and breast cancer markers might be needed, if GATA3 is positive in metastatic carcinoma cells.

Uroplakin II

Uroplakins are a group of transmembrane proteins that are urothelial specific and differentiation-dependent markers and have been shown to be highly specific but with low to moderate sensitivity for urothelial carcinoma [16]. Hoang et al. published their data in 2013 and concluded "The mouse monoclonal uroplakin II antibody (BC21) demonstrated superior sensitivity and specificity in urothelial carcinoma, compared with uroplakin III (BC17 and AU1), suggesting its advantages in the differential diagnosis of urothelial carcinoma and in the detection of tumors of unknown origin" [17].

p63

p63 is a highly sensitive nuclear marker of squamous and urothelial cell neoplasms [18]. However, p63 is not specific; it also stains the myoepithelial cells in the prostate and breast with a rim of nuclear positive myoepithelial cells indicating a noninvasive process. p63 can be used to differentiate between urothelial and prostate carcinomas; it has a similar sensitivity but greater specificity than HMWCK 34βE12 because of nuclear staining which minimizes the nonspecific staining inherent in cytoplasmic stains. However, a special precaution is recommended, since a subset of prostate cancers can be p63 positive. It also needs to be kept in mind that p63 can significantly decrease in high stage and HGUC and virtually absent in micropapillary UC [19].

S100P

S100P or so-called "placental" S100 is another promising marker derived from gene expression-based studies that has been used to confirm urothelial histogenesis, which is a member of the S100 family of calcium-binding proteins [20]. It was initially believed as relatively urothelial

specific, while immunopositivity for S100P has been documented with significant prevalence in several cancers, including significant prevalence of immunopositivity in tumors of the pancreas, breast, colon, lung, desmoplastic melanomas, and ovarian mucinous neoplasms. Notwithstanding this finding, this marker may be of significant value in supporting urothelial differentiation based on its high degree of sensitivity and proven performance in several clinical scenarios [21].

Distinction of High-Grade Prostate Adenocarcinoma from Urothelial Carcinoma

Neoplasms within the prostate and urinary bladder can be primary or result from metastasis or direct extension from adjacent organs. Primary prostate adenocarcinoma can extend up to the urinary bladder, and primary urothelial carcinomas arising either in the urinary bladder or in the urethra can invade into the prostate. The clinical management and prognosis are different for prostate adenocarcinoma and urothelial carcinoma. Hormone therapy is often used to manage patients with advanced prostate adenocarcinoma; chemotherapy is often selected to treat patients with high-stage UC.

Well-differentiated lower-grade UCs and prostate adenocarcinomas can easily be distinguished by histology, and these low-grade tumors do not usually create differential diagnostic problems, but high-grade/poorly differentiated urothelial carcinomas can mimic prostate adenocarcinoma, especially those of Gleason patterns 4 and 5 (score 8, 9, and 10). High-grade adenocarcinoma with solid and papillary growth pattern can mimic high-grade urothelial carcinoma (Fig. 13.4). The possibility of overlapping histologic features, especially in the limited material available from a biopsy specimen, may make it a challenging exercise to accurately distinguish between urothelial and prostate adenocarcinoma. Here we outline a few major morphologic characters on H&E sections and then focus on discussing the utilization of IHC to separate these two different entities.

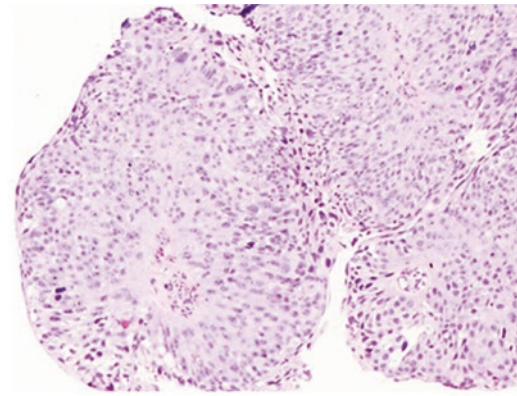

Fig. 13.4 High-grade prostate cancer mimics urothelial carcinoma

Morphologic Characteristics on H&E Sections

High-grade urothelial carcinomas usually show a nesting architecture, squamous differentiation, higher degrees of nuclear pleomorphism, and brisker mitotic activity, compared to poorly differentiated prostate adenocarcinomas which typically show much less nuclear pleomorphism (relatively uniform cells), more prominent nucleoli arranged in infiltrating cords and focal cribriform glands, and lastly, even though high grade, few or no mitoses (Fig. 13.4). However, the morphologic characteristics of the two tumors may overlap, and therefore immunostains may be required to distinguish them.

Commonly Used Immunohistochemical Markers

Numerous immunomarkers expressed on prostatic and urothelial cells have been extensively studied for differentiating between urothelial and prostate adenocarcinomas, including prostate specific antigen (PSA), prostate-specific acid phosphatase (PSAP), prostate-specific membrane antigen (PSMA), prostein (P501s), NKX3.1, cytokeratins (CK7, CK20, and high molecular weight cytokeratins through antibody 34βE12 or CK5/6), uroplakin, thrombomodulin, p63, carcinoembryonic antigen (CEA), GATA3, and many others. [22–25] Different studies delineate the use of different markers for distinguishing between the two neoplasms, and in our practice

we have found that NKX3.1, PSA, PSAP, P501S, thrombomodulin, and 34βE12 are useful markers for this purpose. Other useful markers including GATA3, p63, and Uroplakin II have been discussed in the previous section.

Thrombomodulin and uroplakin are relatively specific for UCs, compared to prostate adenocarcinomas. However, uroplakin has been mentioned to be inconsistent in staining UCs. Thrombomodulin is expressed in UCs though it has a sensitivity ranging from 49 to 91%. This wide variation in sensitivity for thrombomodulin has been ascribed to the varying cutoffs used by different studies with high sensitivity being from studies with any degree of positivity versus lower sensitivities in studies using higher degrees of cutoff for positivity. Whatever the degree of cutoff for positivity, thrombomodulin was not expressed in prostate adenocarcinomas, and therefore it can be used in distinguishing prostate adenocarcinoma from UC [26].

NKX3.1 The homeobox protein NKX3.1 is a transcription factor and tumor suppressor. NKX3.1 has been shown to be highly specific for prostatic origin. Prostate adenocarcinoma and lobular carcinoma of the breast are the only cancers that have been shown to express it. NKX3.1 was expressed from 92% to 97% of high-grade prostatic adenocarcinomas. NKX3.1 proved to be specific and sensitive when differentiating high-grade prostatic adenocarcinomas from poorly differentiated UCs [27–30] (Fig. 13.5).

PSA and PSMA are markers of prostatic epithelium that have been useful in identifying carcinoma of uncertain origin. PSA is expressed in prostate glandular tissue and also in other tissues like breast and salivary gland neoplasms and also anal glands. It is a highly sensitive marker; however, its sensitivity decreases with increasing Gleason score. PSA has a sensitivity ranging from 73% to 97% for poorly differentiated prostate adenocarcinomas. PSMA is like PSA, with a high specificity and a sensitivity of 95–97% in poorly differentiated prostate adenocarcinomas. Since high-grade prostate adenocarcinomas, particularly castration-resistant prostate adenocarcinoma, may be negative for PSA or PSAP, the absence of these markers does not completely exclude a prostatic origin [30–33].

Recent study shows PSMA and NKX3.1 are more sensitive markers than PSA for metastatic prostate adenocarcinoma to the bone following decalcification. We recommend use of PSMA and NKX3.1, rather than PSA, as the IHC markers to confirm metastatic prostate adenocarcinoma to the bone [34].

ERG A nuclear marker ERG (ETS avian erythroblastosis virus E26 oncogene homology) has recently generated interest. Its expression in prostate depends on the TMPRSS2-ERG fusion

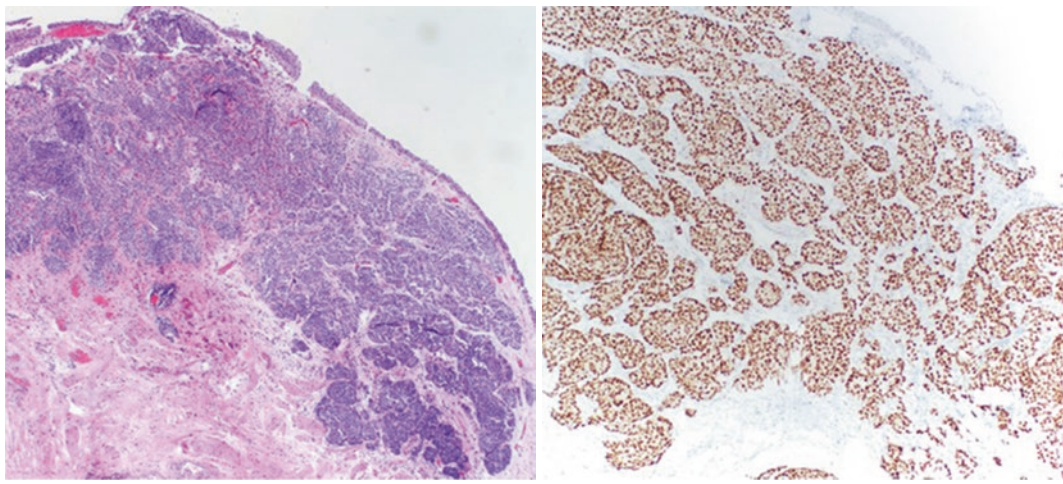

Fig. 13.5 NKX3.1 highlights high-grade prostate adenocarcinoma involving bladder neck

status, which ranges from 2% to 49% sensitivity depending on population studies. It is highly specific for prostate adenocarcinoma and is very helpful when positive in a tumor with the differential diagnoses of UC and prostate cancer. However, ERG has low sensitivity, and high-grade prostate adenocarcinoma, particularly castration-resistant prostate adenocarcinoma, is more likely to be negative [35, 36].

HMWCK Antibody Clone 34βE12 is a specific marker for prostatic glandular basal cells that is directed against cytokeratins CK1, CK5/6, CK10, and CK14. It also stains the urothelium and has a sensitivity ranging from 65% to 100% for UCs, with the variation in sensitivity attributed to be possibly from the antigen retrieval method. With microwave heat retrieval, there was diffuse positivity in all cases of HGUCs as compared to enzyme retrieval methods which showed patchy staining and diffuse positivity only in 65% of UCs. HMWCK in contrast is expressed in up to 11% of prostate adenocarcinomas and has a specificity of 89–97% for UCs. Since prostate cancer can rarely express HMWCK, a precaution is required.

Selection of Immunostain Panel

As we have discussed earlier, the principle of selecting markers should include a complementary panel with speculated positive and negative profile (Table 13.2).

Pitfalls

A very small percent of high-grade prostate adenocarcinomas may lose the expression of PSA; on the other hand, very rarely p63, a marker for UC, can be positive in prostate adenocarcinoma [37]. Therefore, the interpretation of immunostains in this small proportion of cases requires even more caution, and a constellation of features should be used.

Table 13.2 IHC panel for differentiation of urothelial carcinoma from prostate adenocarcinoma

	Urothelial Ca	Prostate Ca
Uroplakin	+	–
GATA3	+	–
P63	+	–
HMWK	+	–
NKX3.1	–	+
PSA	–	+
PSMA	–	+
P501S	–	+
PSAP	–	+

UCs arising from the prostate and direct extension from the urinary bladder share the very same histologic features and immunoprofile. Careful clinical examination of the bladder is essential, and it is directly related to the tumor staging.

A useful algorithmic approach based on our practice is to stain with two or more complementary immunostains that are available in the laboratory, including NKX3.1, PSA, PSMA, P501S, or PSAP and GATA3, HMWK, p63. If it still unresolved, then stain with other markers as needed. Very rarely is the tumor unresolved after these markers.

Nephrogenic Adenoma and Its Mimickers

Nephrogenic adenoma (NA) is a relatively frequent lesion of the urinary tract, which occurs predominantly in the bladder, as well as in the renal pelvis, ureter, and urethra, with a male to female incidence ratio of 2:1. The term nephrogenic metaplasia can be used interchangeably with NA.

Irritative bladder symptoms, occasionally with hematuria, are the usual chief complaints. A well-established association between NA and mucosal trauma (i.e., nephrolithiasis, bladder reconstruction, catheterization, chronic inflammation, intravesical BCG therapy, urinary tract infection, and radiation) led to the once widely accepted conclusion that NA results from a metaplastic response. However, relatively recent studies have shown an association of NA with renal transplant and immunosuppression, and these lesions likely represent an implantation of renal tubular epithelium into a disturbed urothelial mucosa [38].

Cystoscopically, NA is seen as single or multiple papillary, polypoid, mulberry-like, or

shaggy exophytic lesions in the background of an inflamed urothelial mucosa (Fig. 13.6).

Several histologic patterns for NA have been described, including tubular, cystic, polypoid, solid, and very recently flat and their combinations [41]. The epithelium lining these structures is composed of a single- or multi-cellular layer of eosinophilic cuboidal and hobnail cells. Often, the tubules are small and lined by only one cell layer with luminal blue mucin compressing a nucleus, resembling a signet ring cell. However, the presence of prominent basement membrane around these tubules is a useful diagnostic feature for this entity. As for the stroma, it is usually edematous and accompanied by a mixed inflammatory cell infiltrate.

The lining cells of NA are positive for different types of keratin, which have limited value in separating this entity with other malignant tumors, since the latter are of epithelial differentiation as well. However, there are two markers that can be used to diagnose NA.

AMACR: Most NAs (58–78%) are immunoreactive for Alpha-methylacyl-CoA racemase (AMACR), a molecule that is expressed in prostate adenocarcinoma [39] (Fig. 13.7).

PAX2 or PAX-8 is a renal transcription factor that is relatively specific for renal tubular epithelium. We and others reported 100% staining with PAX2 in a series of 39 examples of NA and 100% positive for PAX8 in 15/15 flat pattern nephrogenic adenoma [40, 41] (Fig. 13.7).

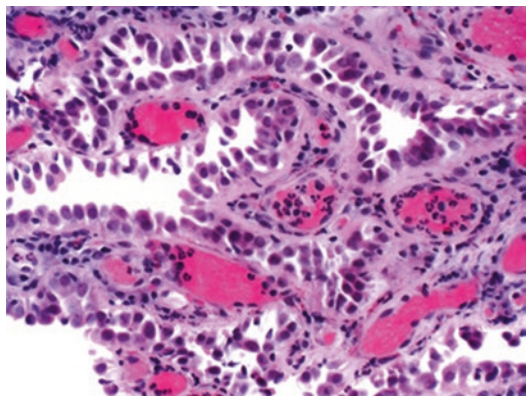

Fig. 13.6 Nephrogenic adenoma mimics urothelial carcinoma or prostate adenocarcinoma

Diagnostic Dilemmas

NA is a benign lesion, with some features mimicking malignant tumors. The major differential diagnoses are those of malignant lesions including clear cell adenocarcinoma, urothelial papillary carcinoma, and prostate adenocarcinoma. The necessity to distinguish NA from the above mimickers cannot be overstated since there are significant differences in management and patient outcome. Whereas patients with NA generally require no further intervention, those with a diagnosis of carcinoma typically undergo transurethral resection, partial or complete cystectomy, and/or adjuvant chemoradiation.

NA vs. Clear Cell Adenocarcinoma of the Bladder

NA is a reactive process and the clustered tubules are most frequently confined to the lamina propria in an inflammatory background. The basement membranes around these tubules are usually

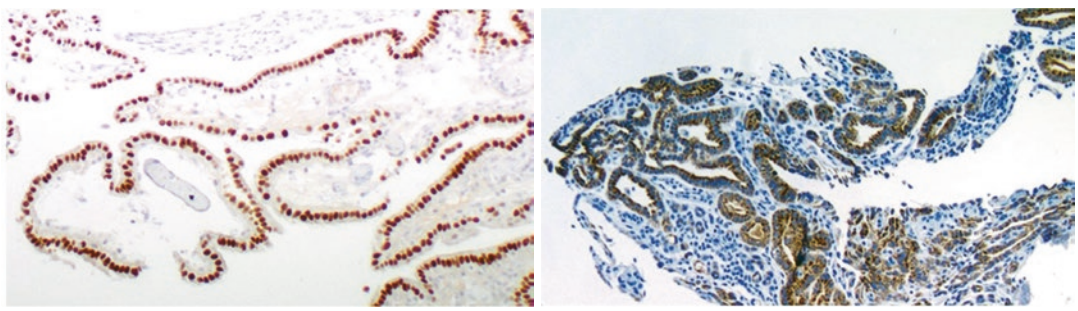

Fig. 13.7 Positive stains of PAX-8 and AMACR in nephrogenic adenoma

well formed, and mitoses are very rare. On the other hand, clear cell adenocarcinoma, like most of the malignant tumors, demonstrates larger size and aggressive histologic features including stromal invasion, anaplastic cytology, high mitotic index, and tumor necrosis.

p53 and Ki-67 are useful in separating these two entities. Clear cell adenocarcinoma shows strong and diffuse p53 nuclear staining, whereas NA shows weak and focal staining. Ki-67 activity is found to be between 10% and 80% in clear cell adenocarcinoma and less than 5% in NA.

Hepatocyte nuclear factor-1β (HNF-1β) is another good but not definitive discriminatory marker in differentiating clear cell adenocarcinomas from NAs. All cases of clear cell adenocarcinomas of the bladder/urethra (n = 18) are positive for HNF-1β, while most of the NAs are negative for HNF-1β [42].

PAX8 is not helpful to differentiate the two lesions because both are immunoreactive for this antibody.

NA vs. Prostate Adenocarcinoma

In male patients, most NAs are found in the bladder neck region and adjacent urethra, which is a frequent site of surgical manipulation in patients with prostate and bladder pathology [43]. The most important diagnostic dilemma when encountering NA in urethral biopsies or TURP from male patients is to distinguish this lesion from prostate adenocarcinoma. It is not uncommon to observe the tubules of NA encroaching deeply into the periurethral stroma of the prostate. Such cases may possibly be misinterpreted as prostate adenocarcinoma.

NA should demonstrate negative staining for NKX3.1, PSA, and PSAP. Prostate adenocarcinoma should stain positively for these markers. Additionally, NA is positive for PAX2/PAX8, whereas prostate adenocarcinoma is negative for both.

AMACR is positive in both NA and prostate adenocarcinoma; immunostains alone can be a potential trap and misleading. Awareness of this pitfall is critical to separate these two lesions; one is benign and the other is malignant.

NA vs. Papillary Urothelial Carcinoma

NA and papillary UC can overlap due to the presence of cellular atypia and papillary growth pattern with fibrovascular cores, which are seen in both entities. Sometimes, there may be a clinical history of papillary UC, and a re-biopsy is performed. We should be cautious not to jump to the conclusion of a recurrence of papillary UC, when we see papillary structures with cytologic atypia. The most important difference is the cell layer: papillary urothelial carcinoma has multiple cell layers, but papillary NA is lined usually by single cell layer.

NA is positive for PAX-2/PAX8, but negative for GATA3 and p63. Conversely UC is positive for GATA3 and p63 and negative for PAX2/PAX8. Again, this complementary panel should be very useful in handling most of the cases within this problematic scenario.

Rarely NA may be associated with various tumors including urothelial neoplasms, prostate or bladder adenocarcinoma, or squamous cell carcinoma of the bladder.

The message is that we should not ignore the associated pathology.

Immunostains for Neuroendocrine Tumors of the Bladder

We have a dedicated chapter for neuroendocrine tumors including small cell carcinoma, carcinoid, and large cell carcinoma with neuroendocrine differentiation. Chapter 9, "Neuroendocrine Tumors of the Urinary Bladder," has concentrated on this important topic.

Distinction of Primary Adenocarcinoma of the Bladder from Secondary Adenocarcinoma Involving the Bladder

If adenocarcinoma is found in the bladder, the key issue for clinical management is whether this is a primary or secondary adenocarcinoma, including metastatic and direct tumor extension from other pelvic organs such as the colon, cer-

vix, or uterus. The treatment of choice for primary adenocarcinoma of the bladder is radical cystectomy or cystoprostatectomy (for male patient). In the case of secondary bladder adenocarcinoma, finding the primary site and administering systemic treatment to control tumor spread will be the focus.

The histologic spectrum of primary urinary bladder adenocarcinoma is wide and should be handled with care. Common histologic patterns are enteric type, which closely resembles colorectal adenocarcinoma histologically and immunohistochemically (CDX2+, CK20+, GATA3-, P63-, CK7-), and non-enteric type, which displays variable histologic and immunohistochemical features, distinct from a colon primary.

If it is enteric type, clinical work-up is necessary to rule out a colonic primary. Coexisting urothelial dysplasia, carcinoma in situ, or invasive urothelial carcinoma strongly favors a primary bladder adenocarcinoma.

Immunostains have limited value in separating primary bladder adenocarcinoma from colorectal adenocarcinoma metastasis or direct extension to the bladder. β-catenin is reported to be the most helpful marker in this setting. More than 90% of colorectal adenocarcinomas demonstrate a strong nuclear positivity, while more than 90% of the primary urinary bladder adenocarcinomas express beta-catenin with a strong membranous pattern [44, 45].

Metastatic Carcinoma of the Urinary Bladder

Usually a clinical history of other primary carcinoma such as breast, colon, and kidney will make us think about the possibility of a metastasis involving the urinary bladder. However, any unusual histology should alert us of the possibility of metastasis to bladder. Communication with the treating physician and a small panel of immunostains can be useful.

We have discussed GATA3, uroplakin II, and p63, and these markers can be used as evidence of urothelial differentiation, although they are not 100% specific. Of note, GATA3 can be positive in UC, breast cancer, and many other tumors, which should caution us to take advantage of additional tools and information in making the final diagnosis.

Again, clinical history and associated urothelial dysplasia or urothelial CIS can be very valuable for the final accurate diagnosis.

Immunohistochemistry in Separating Spindle Cell Neoplasms of the Bladder

The first consideration should be sarcomatoid urothelial carcinoma; we usually are able to appreciate somewhere on the slide a component of conventional urothelial carcinoma or squamous or adenocarcinoma component. An epithelial marker is necessary to confirm this impression. However, in limited specimens without typical areas of recognizable carcinoma, we may need to use immunostains to work up the lesion (Table 13.3).

Immunohistochemistry in Prognosis and Molecular Classification

At present, there is no marker or panel of IHC markers that can be recommended for routine clinical use to prognosticate the clinical behavior

Table 13.3 IHC panel for differentiation of bladder spindle cell lesions

	AE1/3	p63	HMWCK or CK5/6	ALK-1	SMA	Desmin	Myogenin or MyoD1
Sarcomatoid UC	+	+/–	+/–	–	–/+	–	–
IMT	+	–	–	+	+	+/–	–
Leiomyosarcoma	–	–	–	–	+	+	–
Rhabdomyosarcoma	–	–	–	–/+	+	+	+

or to select therapy for urothelial carcinoma, although the need to improve and personalize therapy for this disease is pressing [16]. Predictive biomarkers that are able to forecast and stratify patient response to novel and emerging targeted therapies are also currently sought. Several markers, in varying stages of validation, which have been reported for various prognostic or predictive roles, are briefly described below.

CK20, CD44, Uroplakin, CK14, GATA3, and CK5/6

Recent integrated genomic and protein analysis studies have been used to delineate urothelial carcinoma subgroups. It may help to define subsets of patients who will response and achieve higher survival rates. IHC analysis using limited markers (CK5/6, CD44, CK14, GATA3 and CK20, uroplakin) can fairly subtype urothelial carcinoma into luminal and basal groups [46].

PD-L1

The introduction of immune checkpoint blockade therapy has transformed the management of advanced bladder cancer. The prognostic value of programed death-ligand 1 (PD-L1) in UC has been assessed in several studies, while the results remain controversial and heterogeneous. Despite its limitations, PD-L1 immunohistochemistry may serve as a predictive biomarker of anti-PD-L1/PD1 therapy. Three antibody clones for PD-L1 (SP263, 22C3, and SP142) are considered predictive assays to identify UC patients who are more likely to respond to anti-PD-1/PD-L1 inhibitors, durvalumab, pembrolizumab, and atezolizumab, respectively. Various studies have shown overall good analytical comparability of PD-L1 companion assays and indicate that all three clones are potentially useful in the evaluation of PD-L1 expression in UC [47].

Other promising markers including Ki-67 have potential utility for predicting disease recurrence in noninvasive UC, p53 may have a role in prognostication of progression in muscle invasive disease, and Her2, EGFR, and VEGF may have a role in selection of appropriate therapy [16].

Summary

A few messages need to be emphasized: use the histologic features to formulate a short list of differential diagnoses; and select a panel of immunomarkers which will be expected to be complimentary among the possible entities. Be aware of the staining patterns (expression locations) of each antibody that are applied in the case, taking the clinical history, histology, immunoprofile, and clinical consequences into consideration to render an accurate final diagnosis confidently.

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