Annals of Diagnostic Pathology 17 (2013) 41–44

Contents lists available at SciVerse ScienceDirect

Annals of Diagnostic Pathology

Immunohistochemical markers for the differential diagnosis of nephrogenic adenomas

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ARTICLE INFO

Keywords:
Nephrogenic adenoma
Pax-8
P63
PSA
S100A1
CEA

ABSTRACT

Nephrogenic adenoma (NA) is a rare benign lesion commonly occurring in the urinary bladder that poses challenges to devising a diagnosis. In this study, 21 cases of NAs were studied by performing immunohistochemistry for PAX8, p63, CK903, PSA, S100A1, BerEP4, and CEA on routine tissue sections. PAX8 showed diffuse moderate to strong (2+ and 3+) nuclear staining in all of NAs (n = 6 and 15, respectively) and negative in the normal urothelium (n = 15). Nuclear staining for p63 was not seen in any case of NAs examined (n = 19) and was diffuse and strong (3+) in the normal urothelium (n = 14). High-molecular-weight keratin CK903 showed weak (1+) diffuse staining in all of the NAs examined (n = 19) and diffuse and moderate to strong positivity in the normal urothelium (n = 16). PSA staining was negative in both of the NAs (n = 21) and normal urothelium (n = 16). S100A1 showed strong (3+) diffuse staining in 19 of 20 of the NAs examined (n = 19) and diffuse weak (1+) (n = 14) to moderate (n = 3) staining in the normal urothelium. BerEP4 showed focal to diffuse, mild to moderate (1+ and 2+) cytoplasmic staining in all of NAs (n = 2 and 19) and negative in the normal urothelium (n = 19). CEA staining was negative in both of the NAs (n = 21) and normal urothelium (n = 17). A panel composed of PAX8, p63, PSA, S100A1, and CEA appears to be sensitive and specific in differentiating NA from its mimics of urothelial and prostatic origins.

The resemblance of nephrogenic adenomas (NA) to the developing renal tubules is binding. This lesion was first described by Davis [1] in 1949 and, later in 1950, was first named by Friedman and Kuhlenbeck [2]. Other terms used for NA include *nephrogenic metaplasia* and *adenomatous metaplasia*. Nephrogenic adenomas are rare benign lesions commonly occurring in the urinary bladder in approximately 75% of the recognized lesions. They can be anatomically found dispersed within the bladder wall but can rarely be found in the anterior wall of the urinary bladder. They can also occasionally occur in the urethra, ureter, and renal pelvis. Nephrogenic adenomas are associated with previous urological procedures, instrumentation, chronic inflammation and infections, especially in those treated with intravesical chemotherapy or bacilli Calmette-Guerin. Interestingly enough, approximately 8% of the cases of NAs are found in renal transplant patients. A few cases of NA have been described in children who have had extensive instrumentation, but it more commonly occurs in adults with a male preponderance of 2:1 [3]. Most commonly, the patient's symptomatology consists of gross hematurea, frequency, and dysurea. The cystoscopic appearance of NA is variable. Most have a papillary cystoscopic architecture. They have also been described to be sessile and polypoid. Microscopically, NAs have a tubulocystic or papillary pattern of architecture and, therefore, can mimic many lesions deriving from or metastatic to the urinary bladder. Less commonly, these lesions show a solid or nested growth pattern. Generally, these structures are composed of a single layer of cuboidal or columnar epithelium with scant eosinophilic cytoplasm resembling the epithelial layer of the distal part of the nephron. Lesions with papillary architectures such as urothelial papilloma, papillary urothelium neoplasm with low malignant potential, low-grade papillary urothelial carcinomas, metastatic prostatic adenocarcinoma, urothelial carcinoma with bland histology including the nest variant of urothelial carcinoma, microcystic urothelial carcinoma, and clear cell adenocarcinoma of the urinary bladder. Nephrogenic adenomas are innocuous but can be diagnostically challenging at times [4]. In this study, we evaluated the staining patterns of 21 cases of NAs by PAX8, p63, CK903, PSA, S100A1, BerEP4, and CEA on routine tissue sections and compared that to the staining patterns of each antibody in the literature in an attempt to identify a useful panel for the differential diagnosis in difficult situations in the same laboratory condition.

1. Materials and methods

Twenty-one cases of NAs were retrieved from the archives of the hospital of the University of Pennsylvania. This study was approved by the institutional review board of the University of Pennsylvania.

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http://dx.doi.org/10.1016/j.anndiagpath.2012.05.003

Table 1
Antibodies

Antibody name	Clonality	Dilutions	Manufacturer
P63 (CM163A)	Monoclonal	1:100	Biocare, Concord, CA
PSA (A0562)	Rabbit polyclonal anti-human PSA	1:4500	Dako, Carpinteria, CA
CK903 (ENZ-C34903)	Mouse monoclonal	1:50	Axxora, San Diego, CA
PAX8 (10336-1-AP)	Rabbit polyclonal	1:100	Proteintech, Chicago, IL
S100A1 (NBP1-78271)	Rabbit monoclonal	1:100	Novus, Littleton, CO
CEA (#A115)	Rabbit polyclonal	1:1100	Dako, Glostrup, Denmark
BerEP4 (#M0804)	Mouse monoclonal	1:50	Dako, Glostrup, Denmark

Immunohistochemistry of formalin-fixed, paraffin-embedded tissue was performed using antibodies summarized in Table 1, including antibodies against P63 (Biocare, Concord, CA; CM163A; 1:100), PSA (Dako, Carpinteria, CA; A0562; 1:4500), cytokeratin 903 (Axxora, San Diego, CA; ENZ-C34903; 1:50), PAX8 (Proteintech, Chicago, IL; 10336-1-AP; 1:100), S100A1 (Novus, Littleton, CO; NBP1-78271; 1:100), CEA (Dako, Glostrup, Denmark; #A115; 1:1100) and BerEP4 (Dako; #M0804; 1:50). Heat-induced epitope retrieval for BerEP4 was done in ER1 solution for 20 minutes. No pretreatment was performed for CEA. P63, PSA, cytokeratin 903, CEA, and BerEP4 immunohistochemistry was done on a Leica Bond instrument using the Novocastra Bond Polymer Refine Detection System. PAX8 and S100A1 immunohistochemistry was performed manually using the Dako Envision + Detection System. The intensity of the nuclear staining for PAX8 and p63 and the cytoplasmic staining for CK903 and PSA were evaluated and graded as 0, 1+, 2+, and 3+. The extent of staining was assigned as focal (<25%), nonfocal (25%-75%), or diffuse (>75%).

2. Results

The NAs showed either papillary (Fig. A and G) or infiltrative pattern (Fig. B). The results of immunohistochemical stains were summarized in Table 2. PAX8 showed diffuse moderate to strong (2+ and 3+) nuclear staining in all of NAs (n = 6 and 15) (Fig. C) and negative in the normal urothelium (n = 15). Nuclear staining for p63 was not seen in any case of NAs examined (n = 19) and was diffuse and strong (3+) in the normal urothelium (n = 14) (Fig. D). High-molecular-weight keratin CK903 showed weak (1+) diffuse staining in all of the NAs examined (n = 19) and diffuse and moderate to strong positivity in the normal urothelium (n = 2 and 16). PSA staining was negative in both of the NAs (n = 21) and normal urothelium (n = 16) (Fig. E). S100A1 staining was strong (3+) positive in NAs (n = 19) (Fig. H), weak (1+) positive in one (n = 1), and the urothelium was weak diffuse (1+) in 14 of the cases (n = 14), and moderate staining (2+) was seen in 3 of the cases. BerEP4 showed focal to diffuse, mild to moderate (1+ and 2+) cytoplasmic staining in all of NAs (n = 2 and 19) and negative in the normal urothelium (n = 19). CEA staining was negative in both of the NAs (n = 21) and normal urothelium (n = 17). CEA staining was positive within the umbrella cells or reactive urothelium of 2 cases (n = 2) (Fig. F).

3. Discussion

The origin of NAs is still controversial; however, Mazal et al [5] proposed that NA was an ectopic autotransplant from the kidney based on the observations that reciprocal sex chromosomes in the cells of NAs, that is, Y chromosomes in cells of NA developed in the urinary bladders of female recipients who received a male kidney graft, and X chromosomes, but not Y chromosomes, in such lesions of male recipients of kidneys from female donors. In addition, immunoreactivity for Lotus tetragonolobus agglutinin and Sophora japonica agglutinin, markers characteristically reactive with renal tubular cells, reacted with NAs that developed in the recipients' urinary bladder, lending further support for such hypothesis. Nephrogenic adenomas, though benign, can pose significant challenge in surgical pathology practice. Nephrogenic adenomas can configure as a papillary lesion mimicking papillary cystitis, urothelial papilloma, and papillary urothelium neoplasm with low-malignant-potential, low-grade papillary urothelial carcinomas. Furthermore, NAs involving deep lamina propria and/or superficial muscle can mimic metastatic prostatic adenocarcinoma, urothelial carcinoma with bland histology including nest variant of urothelial carcinoma, and microcystic urothelial carcinoma among the fibers of muscularis mucosa. Nephrogenic adenomas may also show clear cell histology that may be confused with clear cell adenocarcinoma of urinary bladder.

In this study we evaluated a group of markers in NAs to attempt to identify most efficient markers to help in differential diagnosis.

PAX8 is a member of a family of transcription factors playing an important role in the organogenesis for the nephritic cell-lineage [6,7]. It is present in normal and neoplastic tissue of renal origin [8,9]. Pax-8 has also been shown to be positive in 8 of 8 NAs [8]. In this study, we showed strong nuclear positivity in 21 of 21 cases of NA, consistent with previous observation. On the contrary, urothelium is negative for this marker. In cases of a urothelial carcinoma in the differential diagnosis, the pair of markers of Pax-8 and P63 can be used to facilitate the diagnosis.

Nephrogenic adenoma has been reported to be positive for alpha-methylacyl-CoA racemase [10,11], although it has been argued that difference in detection methods may result in positive labeling [12]. In the scenario where NA is found in the prostatic urethra, or the prostatic adenocarcinoma infiltrating the urinary bladder, prostatic adenocarcinoma should be included in the differential diagnosis. In this setting, PIN4 (p63, high-molecular-weight cytokeratin, and P504S) may not be helpful because NAs and prostate adenocarcinoma may stain the same (p63-negative and P504S-positive). However, in most cases, PSA staining can help in distinguishing these two. In our study, all 21 cases are negative for PSA, which may be useful in the difficult situation [10].

In cases where NAs show clear cytoplasm, clear cell adenocarcinoma of the urinary bladder must be at the top of the differential diagnosis [13]. This is a common and difficult predicament in the diagnosis of these lesions because the histologic papillary architecture occurs in both lesions. Although in clear cell adenocarcinoma of urinary bladder a solid pattern predominates and the cytologic atypia is more prominent, the similarities create a pitfall in the diagnosis. In addition, the positivity of clear cell adenocarcinoma for Pax-8 further complicates the matter [9]. Carcinoembryonic antigen (CEA) has been found to be positive in clear cell adenocarcinoma of urethra [14]. Limited study has been performed for CEA in NA [15]. In our study, all 21 cases are negative, which may help to differentiate these two identities in difficult situations. However, morphology evaluation plays a key part in the differentiation of these two lesions. Key morphological features favoring a diagnosis of clear cell adenocarcinoma include more prominent pleomorphism especially hyperchromatic enlarged nuclei, and extensive muscular invasion. Presence of mitoses (>1/10) and a high rate of Ki67 count raise the suspicion for clear cell adenocarcinoma, and clinical correlation, careful follow-up, and re-biopsy may be required if a definitive diagnosis cannot be rendered at times [13,16].

In addition, we also evaluated S100A1 expression in the NA. S100A1 is a calcium binding protein and may show a differential