A Comprehensive Analysis of PAX8 Expression in Human Epithelial Tumors

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Abstract: PAX8 is a paired-box gene important in embryogenesis of the thyroid, Müllerian, and renal/upper urinary tracts, and expression of PAX8 has been previously described in carcinomas from each of these sites. However, a large study including a wide variety of epithelial neoplasms from multiple organ sites other than the thyroid, kidney, or Müllerian system has not been performed. The goal of this study was to evaluate the utility of PAX8 immunostaining based on the evaluation of a wide range of epithelial tumors. PAX8 immunohistochemistry was performed on 1357 tumors (486 tumors in whole-tissue sections and 871 tumors in tissue microarrays, predominantly epithelial) from multiple organs. Only nuclear staining was scored as positive, and tumors were evaluated for the extent and intensity of staining. Western blot analysis with PAX8 was also performed on multiple tumor cell lines. Nuclear PAX8 staining was present in 91% (60 of 66) of thyroid tumors, 90% (158 of 176) of renal cell carcinomas (RCCs), 81% (13 of 16) of renal oncocytomas, 99% (164 of 165) of high-grade ovarian serous carcinomas, 71% (32 of 49) of nonserous ovarian epithelial neoplasms, 91% (10 of 11) of cervical epithelial lesions, and 98% (152 of 155) of endometrial adenocarcinomas. Of the remaining 719 evaluated tumors, only 30 cases (4%), including 12 thymic neoplasms, 3 bladder urothelial carcinomas, 4 lung squamous cell carcinomas, 2 esophageal adenocarcinomas, 1 pancreatic adenocarcinoma, 2 cholangiocarcinomas, 1 ovarian Sertoli-Leydig cell tumor, 1 ovarian sex cord stromal tumor, 3 testicular mixed germ cell tumors, and 1 acinic cell carcinoma, showed at least weak or focal PAX8 positivity. The unexpected finding was diffuse, moderate staining of PAX8 in a subset of thymomas and thymic carcinomas. The 689 remaining tumors, including but not limited to those from the prostate, colon, stomach, liver, adrenal gland, and head and neck, and small cell carcinomas from the lung, cervix, and ovary, were PAX8 negative. PAX8 specificity was confirmed by Western blot analysis, as expression was detected only in ovarian and RCC cell lines. These results show that PAX8 is a highly sensitive marker for thyroid, renal, Müllerian, and thymic tumors. Importantly, all lung adenocarcinomas, breast and adrenal neoplasms, and the majority of gastrointestinal tumors were negative for PAX8. Therefore, PAX8 is an excellent marker for confirming primary tumor site. In a subset of cases, additional markers, including but not limited to thyroid transcription factor-1, RCC, and Wilms tumor-1, may be needed to distinguish between the 3 most common PAX8-positive tumors.

Key Words: PAX8, immunohistochemistry, carcinoma, Müllerian, renal, thyroid, ovarian, thymus

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In addition to morphologic features, immunohistochemistry is routinely used to determine the primary site of many metastatic tumors, especially those that are poorly differentiated or have unusual morphology. Traditionally, the combined use of CK7 and CK20 is helpful in distinguishing lung, breast, Müllerian, or head and neck (CK7 positive, CK20 negative) versus gastrointestinal (CK20 positive, CK7 negative, or CK20 and CK7 positive) primaries. However, these 2 markers alone are neither sensitive nor specific. The use of lineage-specific markers, such as thyroid transcription factor-1 (TTF-1), Wilms tumor-1 (WT-1),[14,32] mammoglobin,[17,26,46] octamer-binding transcription factor 4,[15] and renal cell carcinoma (RCC),[3] provides additional help in narrowing down primary tumor site or cell differentiation; however, there still exist cases in which the low sensitivity of these markers limits their utility.

PAX8 is a member of the paired-box family of genes that is expressed during organogenesis of the thyroid gland, Müllerian tract, and kidney.[7,37,38] PAX8 has also been shown to be expressed in a subset of renal,[44] bladder,[43] and thyroid neoplasms,[36] and in ovarian malignancies.[6,21,26,35] The absence of PAX8 expression in breast carcinoma[35] and malignant mesothelioma[21] is

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especially useful when the differential diagnosis includes ovarian carcinoma. In well-differentiated neuroendocrine tumors, PAX8 may be helpful in determining primary site, and in predicting favorable outcome in a subset of pancreatic endocrine tumors.[25,41] These studies notwithstanding, a single comprehensive evaluation of PAX8 expression across a wide variety of benign and malignant epithelial tumors from multiple organ sites has not been reported.

Therefore, the purpose of this study was to evaluate the utility of PAX8 expression in a large number of neoplasms (predominantly epithelial) using immunohistochemistry and a combination of tissue microarray (TMA) and whole-tissue sections.

MATERIALS AND METHODS

Case Selection

After approval from the Institutional Review Board at Brigham & Women's Hospital, 1357 cases were evaluated using whole-tissue (N = 486) and TMA (N = 871) sections; some of the latter cases have been described elsewhere.[4,8,21,24] Tissues were obtained from the head and neck, lung, breast, thyroid, parathyroid, thymus, adrenal gland, kidney, esophagus, stomach, pancreas, small bowel, large bowel, appendix, testis, ovary, endometrium, cervix, and bladder. The list of individual tumor types from each organ is presented in Table 1. Normal tissues adjacent to neoplasms and also control tissues in the TMAs were evaluated concurrently (Fig. 1).

Immunohistochemistry

Immunohistochemistry was performed using the Envision Plus/Horseradish Peroxidase system (Dako, Carpinteria, CA) and a polyclonal antibody to PAX8 (1:800 dilution; Proteintech Group Inc, Chicago, IL) as previously described.[21] In brief, paraffin-embedded sections were incubated in hydrogen peroxidase and absolute alcohol for 30 minutes to block endogenous peroxidase activity. Antigen retrieval was performed using pressure cooker pretreatment in a citrate buffer (pH = 6.0). Tissue sections were subsequently incubated with the primary antibody for 40 minutes at 25°C. After tris-buffered saline (rinses, the tissue was incubated using the Envision Plus secondary antibody for 30 minutes, followed by diaminobenzidine for 5 minutes. Appropriate positive (tonsil lymphocytes) and negative (incubation with secondary antibody only) controls were stained in parallel for each round of immunohistochemistry. PAX8 was evaluated for nuclear staining only. Immunoreactivity with normal B lymphocytes was used as an internal positive control and as an intensity reference when present.

Western Blot Analysis

Eighteen established renal, ovarian, prostatic, breast, colonic, cervical, and brain tumor cell lines were also used to evaluate PAX8 protein expression. They included OVCAR-3, HeyA8, TOV21G, SKOV3, OVCA433, OVCA420, MCF7, T47D, HeLa, 769P, 786-O, HT29,

TABLE 1. PAX8 Expression in Human Tumors from Multiple Organs

Tumor Type	No. Positive	Total No. Cases (%Positive)
Genitourinary		
RCC		
Clear cell RCC	115	123 (93)
Papillary RCC	19	25 (76)
Chromophobe RCC	4	5 (80)
CDC	1	2 (50)
Xp11 translocation carcinoma	5	6 (83)
Mucinous tubular and spindle cell CA	3	3 (100)
Clear cell tubulopapillary RCC	4	4 (100)
Tubulocystic RCC	1	1 (100)
NOS or metastatic	5	6 (83)
Renal angiomyoadenomatous tumor	1	1 (100)
Angiomyolipoma	0	6 (0)
Oncocytoma	13	16 (81)
Bladder urothelial carcinoma	3	17 (18)
Clear cell adenocarcinoma of bladder/ureter	2	2 (100)
Prostate adenocarcinoma	0	86 (0)
Gastrointestinal		
Gastric ACA	0	22 (0)
Gastric (M)	0	10 (0)
Colon ACA	0	41 (0)
Colon (M)	0	11 (0)
Colon (SQ)	0	2 (0)
Anal (SQ)	0	2 (0)
Appendiceal (M)	0	30 (0)
Gallbladder ACA	0	1 (0)
Cholangiocarcinoma	2	2 (100)
Esophageal ACA	2	8 (25)
Esophageal (M)	0	2 (0)
Esophageal (SQ)	0	7 (0)
Pancreatic ACA	1	12 (8)
Pancreatic (M)	0	4 (0)
Pancreatic (solid pseudopapillary)	0	1 (0)
Hepatocellular carcinoma	0	6 (0)
Breast		
Invasive ductal carcinoma	0	91 (0)
Invasive lobular carcinoma	0	19 (0)
Mixed carcinoma	0	19 (0)
Head and neck		
Squamous cell carcinoma	0	5 (0)
Squamous dysplasia	0	6 (0)
Acinic cell carcinoma	1	3 (33)
Adenoid cystic carcinoma	0	13 (0)
Basal cell adenoma/carcinoma (salivary gland)	0	4 (0)
Mucoepidermoid carcinoma	0	8 (0)
Olfactory neuroblastoma	0	3 (0)
Polymorphous low grade ACA	0	3 (0)
Pleomorphic adenoma	0	4 (0)
Carcinoma ex pleomorphic adenoma	0	1 (0)
Sinonasal adenocarcinoma	0	2 (0)
High grade salivary gland ACA	0	4 (0)
Salivary duct carcinoma	0	5 (0)
Myoepithelial carcinoma	0	2 (0)
Salivary carcinomas/ACA, NOS	0	12 (0)
Atypical carcinoid (larynx)	0	6 (0)
Clear cell odontogenic carcinoma	0	1 (0)
PD carcinoma, NOS	0	3 (0)
Lung		
Adenocarcinoma	0	120 (0)
Squamous cell carcinoma	4	12 (33)
Adenosquamous	0	3 (0)
Carcinoid	0	2 (0)
Small cell carcinoma	0	9 (0)

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TABLE 1. (*continued*)

Tumor Type	No. Positive	Total No. Cases (%Positive)
Ovary		
Serous carcinoma, high grade	164	165 (99)
Mucinous ACA	10	25 (40)
Endometrioid ACA	11	12 (92)
Mixed carcinoma	6	7 (86)
Clear cell carcinoma	2	2 (100)
Transitional	3	3 (100)
Fibroma	0	4 (0)
Sclerosing stromal tumor	0	2 (0)
Germ cell tumor	0	2 (0)
Sertoli-Leydig tumor	1	5 (20)
Granulosa cell tumor	0	7 (0)
Sex cord stromal tumor, NOS	1	2 (50)
Small cell carcinoma	0	7 (0)
Endomyometrium		
Endometrial ACA	152	155 (98)
MMMT	3	5 (60)
Leiomyoma	0	2 (0)
Cervix		
HSIL	2	2 (100)
SCC	2	2 (100)
ACIS	5	5 (100)
Invasive ACA	1	2 (50)
Small cell carcinoma	0	5 (0)
Gestational neoplasms	0	4 (0)
Testicular		
Mixed germ cell tumor	3	7 (43)
Thyroid		
Anaplastic carcinoma	4	5 (80)
Papillary carcinoma	39	39 (100)
PD (insular) carcinoma	3	4 (75)
Follicular carcinoma	10	11 (91)
Follicular adenoma	4	4 (100)
Medullary thyroid carcinoma	0	3 (0)
Thymus		
Thymoma	8	9 (89)
Thymic carcinoma	4	5 (80)
Parathyroid adenoma/atypical neoplasm	5	6 (83)
Adrenocortical		
Adenoma	0	6 (0)
Carcinoma	0	9 (0)

ACA indicates adenocarcinoma; ACIS, adenocarcinoma in situ; CA, carcinoma; HSIL, high grade squamous intraepithelial lesion; M, mucinous; MMMT, carcinosarcoma; NOS, not otherwise specified; PD, poorly differentiated; PSC, serous carcinoma; SCC, squamous cell carcinoma; SQ, squamous.

SW480, PC3, DU145, U251MG, LN229, and IMR90. A majority of these cell lines were obtained from American Type Culture Collection (Manassas, VA) and propagated in Roswell Park Memorial Institute 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen) at 37°C in a 5% CO_2-containing atmosphere, as previously described.[8] The OVCAR-3 cell line was propagated in 1:1 MCDB105 and Media 199 (Sigma-Aldrich, St Louis, MO) with 15% FBS, whereas the 786-O, PC3, DU145, MCF7, and IMR90 cell lines were propagated in Dulbecco's modified Eagle's medium (Cellgro, Herndon, VA) with 10% FBS.

Proteins were extracted using NETN150 (0.5% NP-40, 20 mM Tris pH 8.0, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid) with Protease Inhibitor cocktail 1:100 (Hoffmann-La Roche Ltd, Basel, Switzerland). Protein amounts were quantified using Bio-Rad protein assay (Bio-Rad, Hercules, CA) and 10 μg of total protein was loaded on 4% to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel (Invitrogen, Carlsbad, CA). Proteins were transferred to a nitrocellulose membrane, which was then blotted with a polyclonal rabbit anti-PAX8 antibody (1:1000 dilution; Proteintech, Chicago, IL) and a rabbit polyclonal anti-glyceraldehydephosphate dehydrogenase (GAPDH) antibody (1:2000 dilution; Cell Signaling, Beverly, MA); GAPDH served as a loading control. Both primary antibodies were exposed for 1 hour at room temperature. A horseradish peroxidase (HRP)-linked anti-rabbit IgG secondary antibody (1:4000 dilution; GE healthcare, Bucks, UK) was used for detection (incubation for 45 minutes at room temperature). All blots were developed using HRP Oxidizing and Luminol Solutions (Boston Bioproducts, Worcester, MA) and analyzed on the FlourChem HD2 imaging system (Alpha Innotech, San Leandro, CA).

RESULTS

Results of immunohistochemical staining patterns for PAX8 are summarized in Table 1.

PAX8 Staining in Non-neoplastic Tissues

As expected, strong nuclear staining for PAX8 was seen in follicular cells of the thyroid (Figs. 1A, B), in Müllerian epithelial cells (endometrium, endocervix, secretory cells of the fallopian tube) (Figs. 1C–H), and renal tubular epithelium (proximal convoluted tubules, distal convoluted tubules, and collecting tubules, but not mesangial or epithelial cells of the glomerulus) (Figs. 1I–L). Strong and diffuse staining was also seen in the epithelial lining of the vas deferens (Figs. 1M, N). As previously reported, pancreatic islet cells were immunoreactive for PAX8 (Figs. 1O, P).[25] In contrast, weak patchy staining was observed in a subset of urothelial and squamous lined structures (ureter, bladder, esophagus, and ectocervix) (not shown). Non-neoplastic thymic epithelial cells (but not thymic T lymphocytes) (Figs. 3A, B) and normal to hypercellular parathyroid tissues (Figs. 1Q, R) showed diffuse but weak-to-moderate staining in a subset of cases. In contrast to T lymphocytes, B lymphocytes are consistently immunoreactive with PAX8 (Figs. 1S, T). PAX8 staining was not observed in normal endometrial stroma, myometrium, lung parenchyma, breast parenchyma, endothelial cells, smooth muscle, placental villi, prostatic epithelium, or prostatic stroma.

PAX8 Staining in Benign Tumors and Precursor Lesions

Consistent with previous studies that have shown PAX8 expression in thyroid and renal epithelial neoplasms (Fig. 2, Table 2), this study confirmed PAX8 expression in benign tumors of the thyroid and in a subset of benign kidney tumors, as 4 of 4 (100%) thyroid

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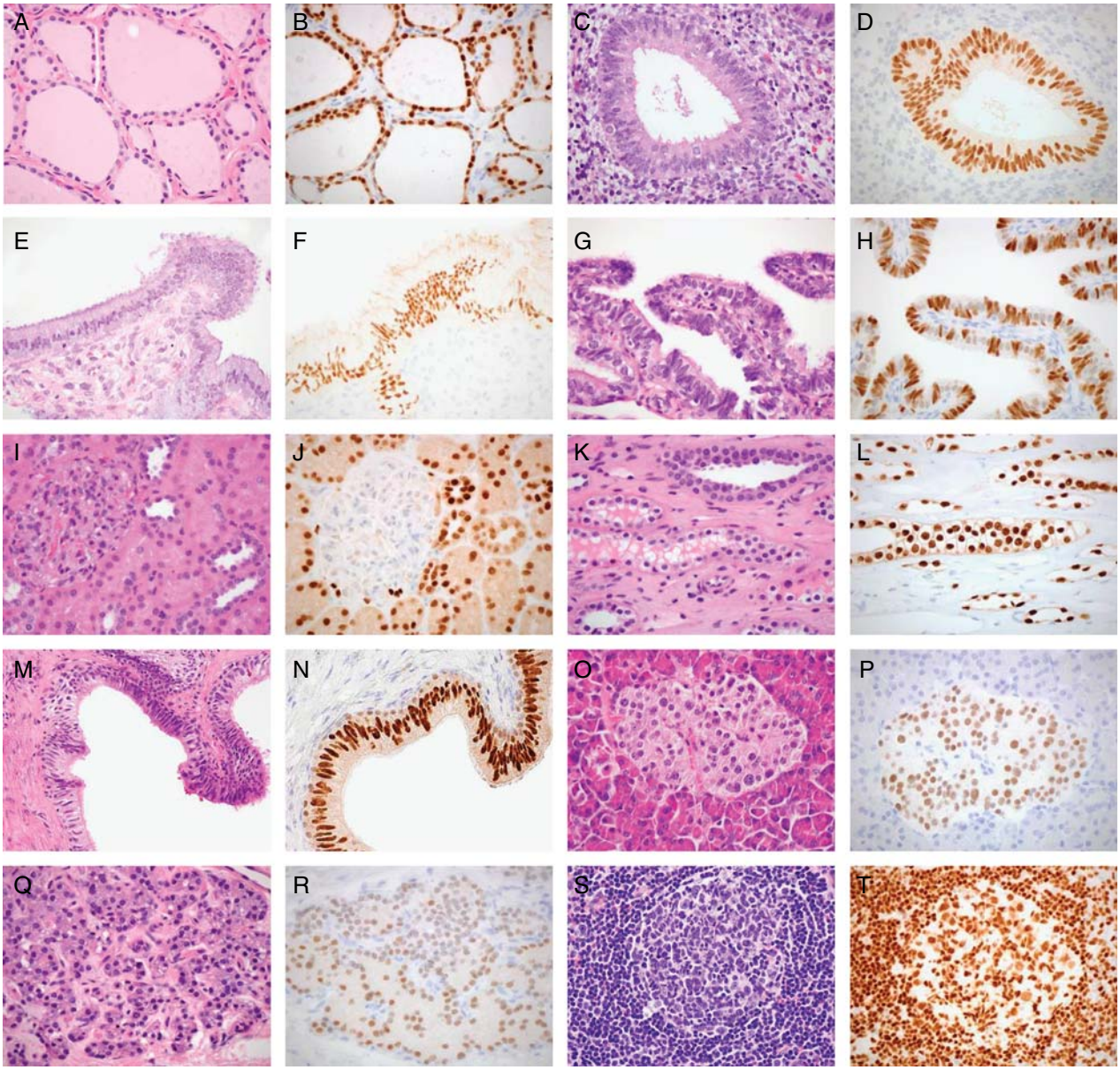

FIGURE 1. PAX8 expression in non-neoplastic human tissues. A and B, Thyroid gland. C and D, Proliferative endometrium; note endometrial stroma is negative for PAX8. E and F, Endocervix. G and H, Fallopian tube fimbria; PAX8 is positive in secretory cells but not in ciliated cells. I and J, Proximal and distal convoluted tubules of the nephron; note the glomerulus is negative for PAX8. K and L, Collecting tubules of the kidney. M and N, Vas deferens epithelium is strongly positive for PAX8. O and P, Pancreatic islet cells. Q and R, Parathyroid gland; cells are weakly positive for PAX8. S and T, B lymphocytes are strongly positive and can act as an internal control in almost any tissue.

follicular adenomas and 13 of 16 (81%) oncocytomas were strongly and diffusely immunoreactive. All renal angiomyolipomas (N = 6), salivary gland pleomorphic adenomas (N = 4), adrenal cortical adenomas (N = 6), and ovarian fibromas (N = 4) were completely negative for PAX8. Five of 6 (83%) parathyroid neoplasms (Fig. 3A) were immunoreactive for PAX8; however, most showed only a weak multifocal staining pattern, with strong diffuse staining in just 1 case. In situ high-grade squamous (HSIL) (Fig. 3B) and glandular (ACIS) lesions of the cervix (N = 2 and 5, respectively) were moderately to strongly immunoreactive for PAX8.

PAX8 Staining in Malignant Tumors

As expected, nuclear staining for PAX8 was strongest in carcinomas from the thyroid, kidney, and Müllerian tract (Fig. 2). More specifically, PAX8 staining was present in 91% (60 of 66) of thyroid tumors, 90% (157 of 175) of RCCs, 98% (186 of 189) of nonmucinous ovarian epithelial carcinomas, and 98% (152 of 155) of

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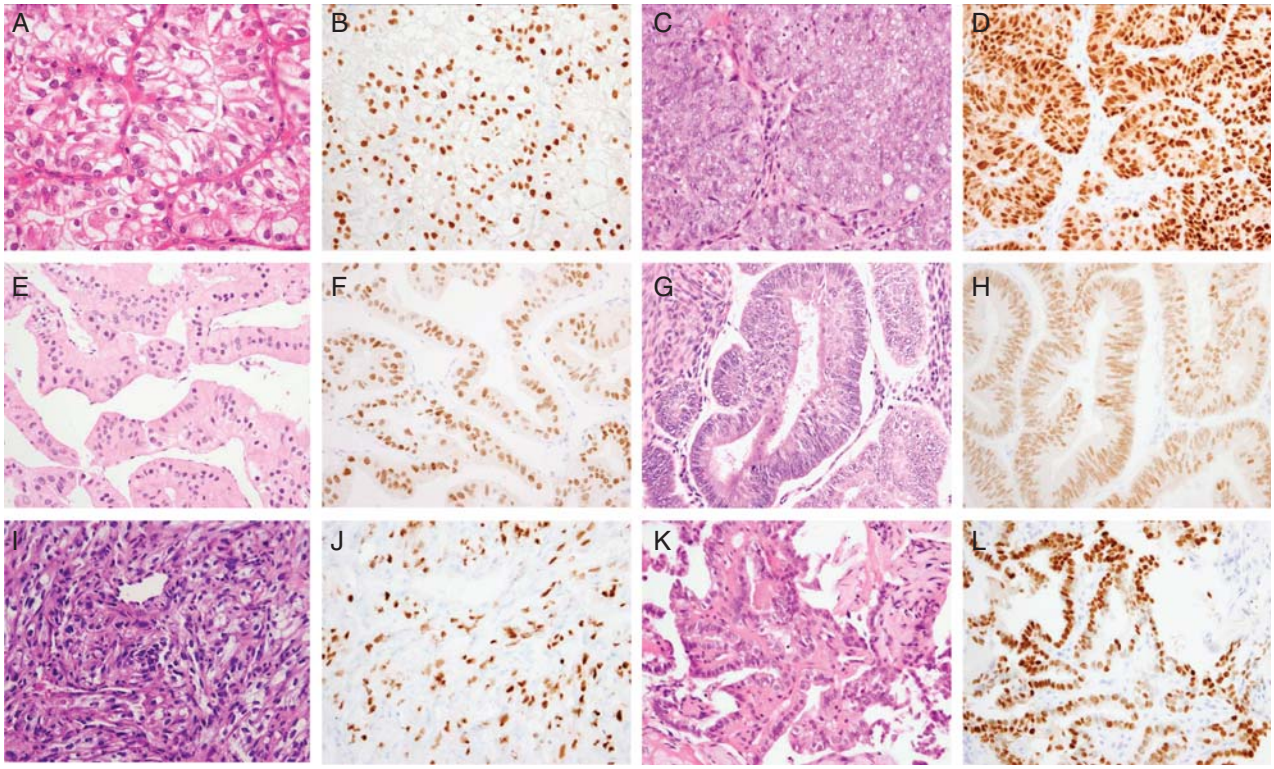

FIGURE 2. PAX8 expression in carcinomas from the kidney, ovary, endometrium, and thyroid. Diffuse, strong nuclear PAX8 expression is seen in most RCCs including, CC-RCCs (A, B), papillary RCCs (E, F), and RCCs with sarcomatoid differentiation (I, J). In addition to serous ovarian carcinoma (C, D), PAX8 is also diffusely expressed in endometrioid carcinomas in the endometrium (G, H). A papillary thyroid carcinoma is strongly positive for PAX8 (K, L).

endometrial adenocarcinomas. There was no obvious trend based on tumor grade in renal and Mullerian carcinomas as the majority of both low-grade and high-grade tumors of various subtypes were positive for PAX8. For example, PAX8 was expressed in 115 clear cell RCCs (CC-RCCs), including 3 Fuhrman nuclear grade (FNG) 1, 79 FNG 2, 14 FNG 3, and 19 FNG 4. Of the 18 RCCs that were negative for PAX8, subtype and FNG were as follows: one CC-RCC FNG 1, 6 CC-RCCs FNG 2, 1 CC-RCC FNG 3, 3 PRCCs FNG 2, 2 PRCCs FNG 3, 1 PRCC FNG 4, 1 chromophobe-RCC FNG 2, 1 translocation (Xp11)-RCC FNG 3, 1 collecting duct carcinoma (CDC), and 1 high-grade metastatic RCC. Similarly in the Mullerian tract, endometrial adenocarcinomas that were positive for PAX8 included 79, 45, and 11 cases of grades 1, 2, and 3 endometrioid adenocarcinomas, respectively, and 3 high serous carcinomas, 1 clear cell carcinoma, plus 13 high-grade carcinomas of mixed endometrioid and serous type. The 3 cases negative for PAX8 included 1 grade 2 and 2 grade 3 endometrioid adenocarcinomas. In addition, 3 uterine carcinosarcomas showed focal staining for PAX8.

In the ovary, nearly all high-grade (164 of 165) and low-grade[21] serous carcinomas were positive for PAX8. In addition, 20 of 22 (91%) nonserous/nonmucinous ovarian epithelial carcinomas (ie, endometrioid, clear cell, transitional, or mixed) were immunoreactive for PAX8, including 11 grade 1 carcinomas, 2 grade 2 carcinomas, and 7 grade 3 carcinomas; the 2 cases negative for PAX8 included a grade 3 endometrioid carcinoma and a grade 2 mixed endometrioid/mucinous carcinoma. In the cervix, 1 of 2 cervical adenocarcinomas (Fig. 3C) and 2 of 2 squamous cell carcinomas were positive for PAX8, although the latter showed only weak-to-moderate intensity. Among mucinous neoplasms, 40% (10 of 25) of primary well-differentiated ovarian mucinous tumors were positive for PAX8 (Fig. 3D), whereas none of the 57 primary gastrointestinal mucinous tumors from the appendix, colon, stomach, esophagus, or pancreas showed any reactivity for PAX8.

Unexpectedly, primary tumors of thymic origin were nearly always positive for PAX8 (Fig. 4), albeit, slightly less intense when compared with internal control B lymphocytes, and Müllerian, renal, and thyroid tumors. Nine thymomas were evaluated with PAX8: three type A thymomas (Figs. 4C, D), 5 type B thymomas (Figs. 4E–H), and 1 multinodular thymoma (Figs. 4I, J); all except 1 type A thymoma were immunoreactive with PAX8. A more variable staining pattern was observed with thymic carcinomas (Figs. 4K, L), with a complete absence of staining in 1 case, multifocal weak staining in 1 case, multifocal strong staining in 1 case, and diffuse strong staining in 2 cases.

Of the remaining 705 malignant tumors evaluated, only 18 cases (1.9%) showed weak and/or focal staining for PAX8 (Table 1). These 18 cases included 3 of 17 bladder

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TABLE 2. Previously Published Series of PAX8 Expression in Surgical Pathology Specimens

	Albadine et al[1]	Argani et al[2]	Fujiwara et al[13]	Kobel et al[19]	Laury et al[21]	Long et al[25]	Lotan et al[26]	Nonaka et al[35,36]*	Sangoi et al[41]	Tong et al[43,44]*	Total (%)
Mullerian											
Pelvic†											
PSC‡			2/2	167/212	161/162		11/11	91/94		22/24	454/505 (90)
Ovary											
SBT					92/92						92/92 (100)
EMOID				48/125				18/21		4/5	70/151 (46)
CCC				100/132				12/12		3/3	115/147 (78)
MMMT										2/2	2/2 (100)
MUC CA				2/31				1/12		0/2	3/45 (7)
Endometrium											
EMOID										49/49	49/49 (100)
PSC										2/2	2/2 (100)
MMMT										1/1	1/1 (100)
Cervix											
ACA										0/5	0/5 (0)
Genitourinary											
Renal											
CC-RCC		5/7						5/5		58/59	68/71 (96)
P-RCC		60/6								19/21	25/27 (93)
Ch-RCC										9/11	9/11 (82)
Tr-RCC		16/21									16/21 (76)
RCC-SD										5/7	5/7 (71)
RCC-NOS			8/8								8/8 (100)
Med CA										2/2	2/2 (100)
CDC	21/21										21/21 (100)
WT								2/2			2/2 (100)
Onco										21/22	21/22 (95)
Urothelium											
UTUCA§	3/34									4/19	7/53 (13)
Bladder							0/13	0/5		0/40	0/58 (0)
Prostate											
ACA			0/1					0/6			0/7 (0)
GCT											
Seminoma								2/3			2/3 (67)
Endocrine											
Thyroid											
Pap CA								17/17			17/17 (100)
FA and FC								18/18			34/34 (100)
Insular CA								7/7			7/7 (100)
Anapl CA								22/28			22/28 (79)
Med CA								6/8			6/8 (75)
NET											
Pancreas						51/81			49/66		100/147 (68)
Ileum						0/47			0/31		0/78 (0)
Appendix									1/11		1/11 (9)
Other Tubular GI						21/42			13/45		34/87 (39)
Lung‖								0/4	0/21		0/25 (0)
Pulmonary											
Lung											
ACA							0/6	0/114			0/120 (0)
SCC								0/29			0/29 (0)
NOS			0/1								0/1 (0)
Gastrointestinal											
Stomach											
ACA								0/5			0/5 (0)
Esophagus											
SCC								0/6			0/6 (0)
Colorectal											
ACA			0/2					0/8			0/10 (0)
HepBil											
CholangioCA			0/2								0/2 (0)
HCC								0/6			0/6 (0)
Pancreas											
ACA								0/5			0/5 (0)
SPP									4/15		4/15 (27)
ACC									0/5		0/5 (0)

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TABLE 2. (*continued*)

	Albadine et al[1]	Argani et al[2]	Fujiwara et al[13]	Kobel et al[19]	Laury et al[21]	Long et al[25]	Lotan et al[26]	Nonaka et al[35,36]*	Sangoi et al[41]	Tong et al[43,44]*	Total (%)
Other											
Breast											
IDC and ILC			0/8				0/16	0/263			0/287 (0)
Mesothelium											
Pleural MM					0/24						0/24 (0)
Peritoneal ML					5/50						5/50 (10)
Skin											
Adnexal ACA and SCC			0/7					0/4			0/11 (0)

*Combined cases from 2 publications.
†Includes tubal, ovarian, and primary peritoneal serous carcinoma.
‡Includes low-grade and high-grade serous carcinoma.
§Includes renal pelvis and ureter.
‖Includes carcinoid, atypical carcinoid, and large cell carcinoma.
ACA indicates adenocarcinoma; ACC, acinar cell carcinoma; Anapl, anaplastic; CA, carcinoma; CCC, clear cell carcinoma; CC-RCC, clear cell renal cell carcinoma; CDC, collecting duct carcinoma; Cholangio CA, cholangiocarcinoma; Ch-RCC, chromophobe RCC; EMOID, endometrioid carcinoma; FA, follicular adenoma; FC, follicular carcinoma; GCT, germ cell tumor; HCC, hepatocellular carcinoma; HepBil, hepatobiliary; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; Med, Medullary; MMMT, carcinosarcoma; MUC, mucinous; NET, neuroendocrine tumors; NOS, not otherwise specified; Onco, oncocytoma; Pap CA, papillary thyroid carcinoma; Peritoneal ML, peritoneal mesothelial lesions; Pleural MM, pleural malignant mesothelioma; P-RCC, papillary RCC; PSC, papillary serous carcinoma; RCC, renal cell carcinoma; SBT, serous borderline tumor; SCC, squamous cell carcinoma; SD, sarcomatoid differentiation; SPP, solid pseudopapillary tumor; Tr-RCC, translocation (Xp11) renal cell carcinoma; UTUCA, upper tract urothelial carcinoma; WT, Wilm's tumor.

urothelial carcinomas, 4 of 12 lung squamous cell carcinomas (Fig. 3E), 2 of 8 esophageal adenocarcinomas, 1 of 12 pancreatic adenocarcinomas, 2 of 2 cholangiocarcinomas (Fig. 3F), 1 of 5 ovarian Sertoli-Leydig cell tumors (Fig. 3G), 1 of 2 ovarian sex cord stromal tumors not otherwise specified (Fig. 3H), 3 of 7 testicular mixed germ cell tumors (Fig. 3I), and 1 of 3 salivary gland acinic cell carcinomas. All remaining 687 tumors, including but not limited to those from the prostate, colon, stomach, liver, adrenal gland, mediastinum (ie, nuclear protein in testis/NUT midline carcinoma),[12] and head and neck, and small cell carcinomas from the lung, cervix, and ovary, were PAX8 negative (Table 1).

Western Blot Analysis

Consistent with our human tissue analysis, Western blot analysis of cancer cell lines showed that PAX8 expression is largely restricted to ovarian and renal carcinoma cells (Fig. 5); thyroid cell lines not tested. Among the ovarian cell lines, OVCAR3, HeyA8, SKOV3, and OVCAR433 serous carcinomas and TOV21G clear cell carcinoma express readily detectable levels of PAX8, whereas the OVCAR420 serous carcinoma cell line expresses marginally detectable levels. The renal cell lines 769P and 786O express readily detectable levels of protein. None of the other examined cell lines showed any detectable levels of PAX8, including breast (MCF7 and T47D), cervix (HeLa), colon (HT29 and SW480), prostate (PC3 and DU145), glioblastoma (U251MG and LN229), and fibroblast (IMR90).

DISCUSSION

There exist 9 members in the *Pax* gene family, all of which contain a paired-box, DNA-binding domain of 128 amino acids at the amino terminus, which is conserved among many species. Distinction among the different *Pax* genes is dependent on the presence or absence of octapeptide-coding regions and a paired-type homeobox.[28] Individual members of the *Pax* gene family have been shown to be crucial for morphogenesis, organogenesis, cell differentiation, and/or tumorigenesis by their corresponding protein products, which act to regulate transcription.[5,31,33]

PAX8 is a paired-box gene that has garnered considerable interest in both clinical and research arenas because of its expression in tumors of the thyroid, kidney, and Müllerian systems (Table 2). In the thyroid, PAX8-PPARγ rearrangement has been shown to occur in approximately 30% of follicular thyroid carcinomas, but not in other subtypes of thyroid carcinoma.[10–12,20,34] In contrast, clinical studies have shown that PAX8 protein expression is present in all papillary and follicular carcinomas, whereas only a subset of anaplastic and medullary carcinomas is positive (Table 2).[36] The coexpression of TTF-1 and PAX8, especially in a metastatic site, is diagnostic of a primary thyroid tumor and excludes a TTF-1-expressing lung adenocarcinoma.

In the kidney, PAX8 expression has been previously demonstrated in both benign and malignant tumors, including clear cell, papillary, chromophobe, and translocation (Xp11.2) RCCs, and in CDCs and oncocytomas (Table 2).[1,2,44] To avoid potential pitfalls, one should be aware that PAX8 can be expressed in a subset of renal pelvis urothelial carcinomas and in clear cell adenocarcinomas of the bladder, but not in conventional urothelial carcinomas in the ureter or bladder (Table 2).[43,44] Coexpression of p63 with PAX8 in a tumor situated in the renal pelvis or medulla is supportive of urothelial carcinoma over a tumor arising from the renal parenchyma[1]; the latter would be expected to express both the RCC antigen (RCC) and PAX8 in approximately 60% to 70% of primary renal epithelial neoplasms (RCC antigen is more specific, but less sensitive compared with PAX8).[3,29]

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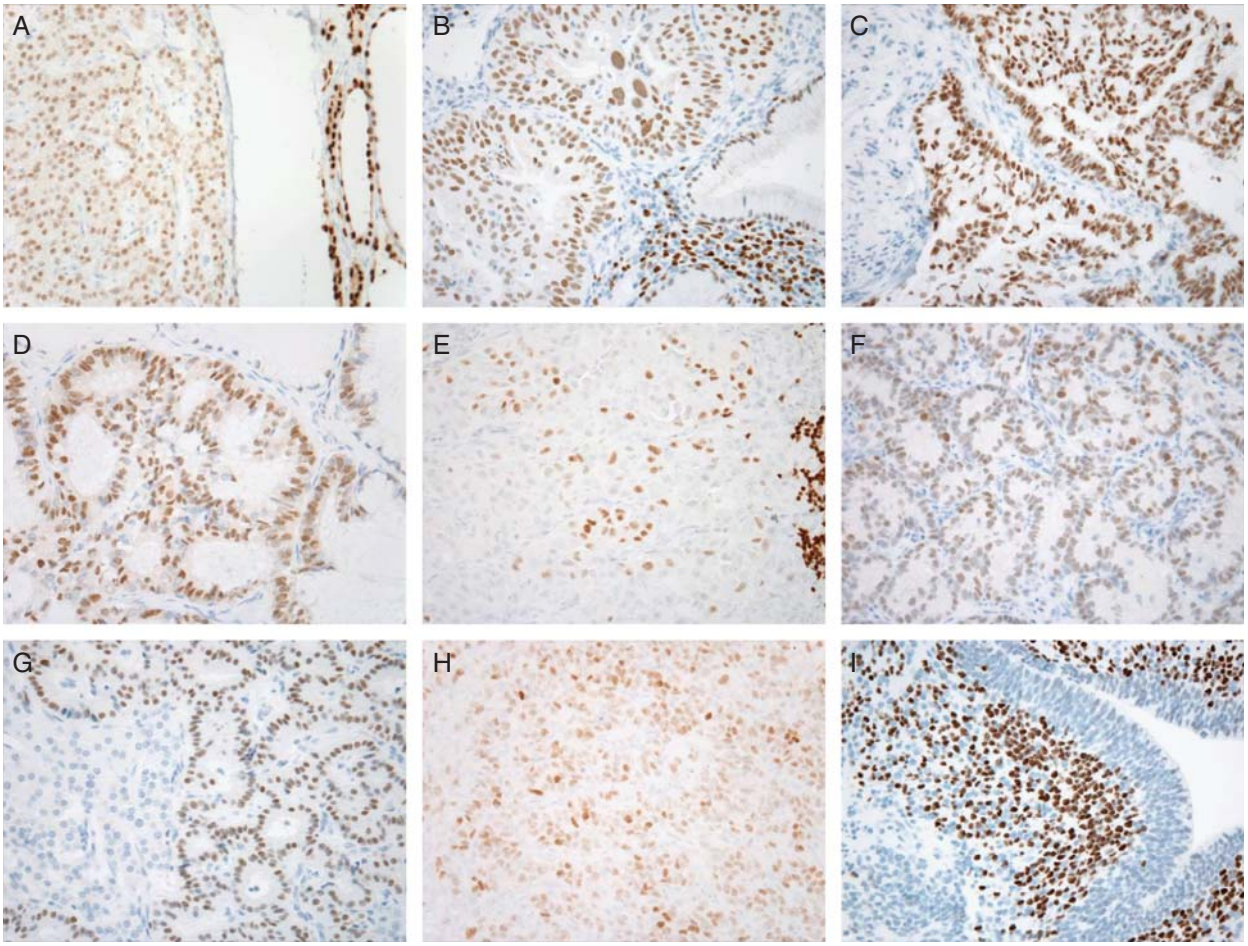

FIGURE 3. Expression of PAX8 in neoplasms from various sites. A, An atypical parathyroid neoplasm shows moderate, yet diffuse immunoreactivity for PAX8; note a small amount of strongly immunoreactive thyroid parenchyma on the right of the image. B, Moderate staining of PAX8 in a high-grade squamous intraepithelial lesion of the cervix; note strongly immunoreactive B lymphocytes in the lower right corner. C, Invasive endocervical adenocarcinoma. D, Primary mucinous ovarian carcinomas are immunoreactive in a subset of cases (approximately 40%), whereas all mucinous gastrointestinal carcinomas were negative (not shown). E, Squamous cell carcinoma of the lung with scattered PAX8-positive tumor cells; note positive B lymphocytes on the right side of the image. F, Two cholangiocarcinomas were multifocally positive for PAX8, albeit with a weak-to-moderate staining pattern. G, Sertoli-Leydig cell tumor of the ovary; note that only sertoli cells were immunoreactive for PAX8, not Leydig cells. H, An ovarian sertoli cell tumor, not otherwise specified, was weakly, but diffusely positive for PAX8. I, In a mixed germ cell tumor of the testis, multiple foci of immature teratomatous elements were strongly positive for PAX8; rare cells from embryonal carcinoma and yolk sac tumor were also positive.

PAX8 expression in the Müllerian system has received the greatest amount of recent attention (Table 2). For one, unlike the thyroid and kidney, which express specific lineage markers, no specific transcription factor has previously been identified in normal Müllerian epithelia. In this regard, the secretory cells of the fallopian tube are strongly positive for PAX8 (Figs. 1G, H).[6,23] In addition, multiple studies have shown that these PAX8-positive fallopian tube secretory cells are the likely source for many ovarian/pelvic serous tumors.[9,18,22,39] Clinically, PAX8 has been shown to be useful in distinguishing serous ovarian carcinoma from breast carcinoma,[35] malignant mesothelioma,[21] and primary adnexal tumors (Table 2).[13] In addition, PAX8 has been shown to be useful as a marker of Mullerian differentiation in serous effusions.[30,42]

Although the individual studies discussed above have shown high sensitivity and specificity for tissues and tumors of the thyroid, renal, and Müllerian tracts, no single comprehensive study has addressed the utility of PAX8 across a large number of epithelial tumors from multiple organs throughout the body. Our current study confirms that PAX8 is a highly sensitive marker for ovarian serous and thyroid carcinomas and that it is also expressed in a significant proportion of renal, thymic, and other Müllerian tumors (Table 1, Figs. 2, 4). In addition, these findings were substantiated by Western blot analysis, which showed that PAX8 expression was found

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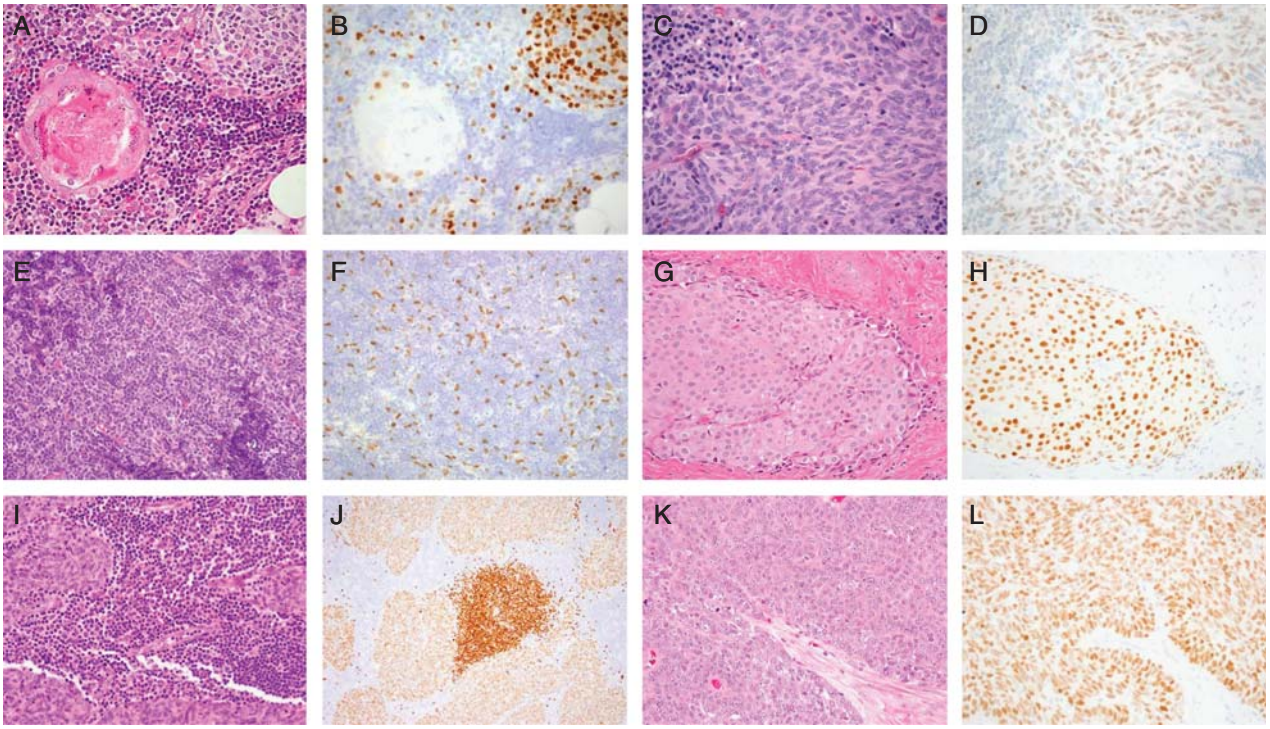

FIGURE 4. PAX8 expression in non-neoplastic and neoplastic thymic tissues. A and B, Non-neoplastic thymic tissue shows PAX8 in thymic epithelial cells and B lymphocytes (upper right corner), but not in T lymphocytes. C and D, A type A thymoma was multifocally and moderately immunoreactive with PAX8. E and F, Scattered PAX8-positive neoplastic thymic epithelial cells are seen in a type B2 thymoma; T lymphocytes are negative. G and H, A type B3 thymoma is strongly and diffusely positive for PAX8. I and J, In the rare multinodular thymoma, neoplastic thymic epithelial cells are positive for PAX8 to a lesser extent than the B lymphocytes; T lymphocytes are negative. K and L, A thymic carcinoma is diffusely immunoreactive for PAX8.

in tumor cell lines from the ovary and kidney, but not in brain, breast, prostate, and colon cell lines (Fig. 5).

Overall, 91% of thyroid neoplasms in this study were immunoreactive for PAX8 (Table 1). Consistent with previously published reports (Table 2), sensitivity for PAX8 was greatest in follicular adenomas, follicular carcinomas, and papillary carcinomas (each 100%), whereas fewer of the poorly differentiated (insular and anaplastic) thyroid carcinomas were immunoreactive for PAX8 (75% and 60%, respectively). None of the thyroid medullary carcinomas were positive for PAX8 in this study, which differs from the study by Nonaka et al[36]; however, it should be noted that in the latter series the majority of cases were only focally positive with $< 25\%$ immunoreactive tumor cells.

Both benign and malignant renal epithelial neoplasms in this study were immunoreactive for PAX8 (Table 1, Fig. 2), with positive cases ranging from 76% to 100% positive (Table 1). Of note, all renal angiomyolipomas were negative for PAX8. These findings showed that, although PAX8 can be useful for confirming a primary kidney tumor, it cannot be used to differentiate the subtype of renal epithelial neoplasm, including CDCs and even renal pelvis urothelial carcinomas (Tables 1 and 2).[1,2,44] Additionally, the absence of PAX8 in a small subset of RCCs (N = 18) cannot be accounted for based on RCC subtype or FNG, as those that were negative included clear cell, papillary, chromophobe, and translocation RCCs of varying FNG (2, 3, or 4). In this series, sampling error may account for at least some of the negative RCCs, as most of these were evaluated by tissue array.

PAX8 expression in tumors of the Müllerian system is less straightforward. Multiple studies (including this study) have shown that nearly all ovarian clear cell carcinomas and low- and high-grade serous tumors from the ovary, fallopian tube, and peritoneum are immunoreactive for PAX8.[19,21,35,40] However, fewer studies have addressed PAX8 staining patterns in nonserous ovarian tumors, the endometrium, and cervix. In this study, we show that the presence of PAX8 in a mucinous carcinoma in the ovary (Fig. 3D) supports the diagnosis of a primary ovarian tumor over a metastasis from the gastrointestinal tract, as none of the latter was immunoreactive for PAX8. Nevertheless, as the sensitivity of this marker in primary mucinous ovarian carcinomas is relatively low (40%), the absence of PAX8 does not entirely exclude an ovarian primary. In the ovary and endometrium, a large subset of endometrioid carcinomas were positive for PAX8 (92% and 98%, respectively) (Figs. 2G, H), and it is our experience that the higher the tumor grade, the more likely it is to stain diffusely and strongly with PAX8. To our knowledge, this study is also the first to report PAX8

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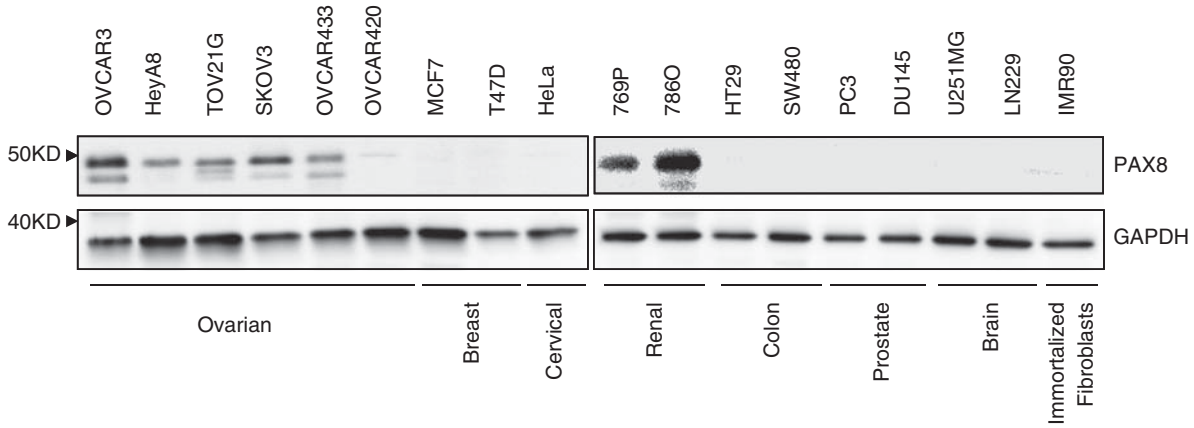

FIGURE 5. PAX8 protein expression analysis in ovarian and nonovarian cell lines. Protein was extracted from 18 cell lines and PAX8 expression level was analyzed by Western blot analysis. PAX8 protein was expressed in cancer cell lines from the ovary and kidney, but not from the breast, cervix, colon, prostate, or brain. GAPDH levels were measured as protein loading control.

expression in conventional (ie, non small cell) in situ and invasive carcinomas of the cervix (Figs. 3B, C).

Interestingly, there was a subset of non-thyroid, -kidney, and -Müllerian tumors that were also immunoreactive with PAX8. The most interesting of these was PAX8 expression in thymic and parathyroid tissues and neoplasms. It is well known that PAX8 is expressed early in development in the thyroid primordium, thyroglossal duct cells, and in the ultimobranchial body, and that PAX8 mutant mice are hypothyroid.[16,27,45] In contrast, these studies did not show PAX8 expression in the third and fourth pharyngeal pouches, which ultimately give rise to inferior parathyroid glands and thymus, and superior parathyroid glands, respectively. Nevertheless, our current study clearly shows expression of PAX8 in these adult organs and associated neoplasms. The most consistent expression was present in thymomas, where the neoplastic epithelial cells were diffusely and moderately immunoreactive with PAX8 (Fig. 4).

Other important findings in this study include the confirmation of previously published series that have shown an absence of PAX8 staining in all lung and breast adenocarcinomas (Tables 1 and 2), two tumors that are frequently in the differential diagnosis with renal and Müllerian carcinomas at metastatic sites. Adrenal cortical and hepatocellular carcinomas can show morphologic overlap with RCC, and these tumors, and benign adrenal adenomas, were shown in this study to be negative for PAX8, making it a useful marker to help distinguish among retroperitoneal carcinomas of unknown primary site.

The absence of PAX8 reactivity in small cell carcinomas from various sites, including the lung, gastrointestinal tract, cervix, and ovary, is an additional interesting finding, especially as a subset of well-differentiated neuroendocrine tumors from similar sites have previously been shown to be positive for PAX8.[25,41] More specifically, pancreatic endocrine tumors and appendiceal and rectal carcinoid tumors are most frequently positive for PAX8, whereas ileal and pulmonary carcinoid tumors are negative. Furthermore, PAX8-negative pancreatic endocrine tumors are more likely to be associated with liver metastases, suggesting that PAX8 may represent a prognostic marker for this tumor type.

Overall, the findings in this study verify that PAX8 nuclear expression is an extremely useful tool in clinical practice; however, as with any immunohistochemical stain, the results should not be evaluated in isolation or without clinical context, and hematoxylin and eosin morphology must always be considered. As with all immunostains, occasional unexpected results (ie, pitfalls) may occur, as was the case with $< 3\%$ of cases (excluding the thymic neoplasms) in this series, which showed focal to multifocal expression of PAX8. The significance of PAX8 expression in this small subset of tumors is unclear, but it does show that no marker is entirely specific for a particular tumor type. Nevertheless, this study confirms that, in the great majority of clinical cases, PAX8 expression is highly sensitive and useful to confirm primary and metastatic tumors that arise in the kidney, thyroid, Müllerian tract, and the thymus. In a subset of cases, additional markers such as TTF-1, RCC, p63, and WT-1 may be needed to distinguish among the 3 most common PAX8-positive tumors.

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