GATA3 Is a Sensitive and Specific Marker of Benign and Malignant Mesonephric Lesions in the Lower Female Genital Tract

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Abstract: GATA3 is a transcription factor critical for embryogenesis, development, and cell differentiation. Recent studies have suggested that GATA3 is a sensitive and relatively specific biomarker for urothelial and breast carcinomas, with most Müllerian carcinomas being negative. We investigated GATA3 expression in mesonephric/Wolffian remnants and tumors in the female genital tract. A western blot was performed to assess specificity for the GATA3 antibody. GATA3 immunohistochemistry was performed on 59 formalin-fixed paraffin-embedded mesonephric samples, including 17 mesonephric remnants (MR; 11 cervical and 6 fallopian tube), 15 mesonephric hyperplasias, 21 mesonephric carcinomas, and 6 female adnexal tumors of probable Wolffian origin. Thirty conventional endocervical adenocarcinomas (ENDO-CA), 9 gastric-type cervical adenocarcinomas, and 165 endometrial adenocarcinomas (EM-CA) were also evaluated. GATA3 nuclear intensity and extent of staining was evaluated. The western blot revealed GATA3 expression in seminal vesicle and cell lines derived from breast and urothelial carcinomas, but not in other cell lines including ovarian, cervical, and endometrial cancers. All cervical MRs and mesonephric hyperplasias, 5/6 (83%) fallopian tube MRs, and 20/21 (95%) mesonephric carcinomas were GATA3 positive, although with great variability in both intensity (weak to strong) and extent (1+ to 3+) of staining. Only 1/6 (17%) female adnexal tumors of probable Wolffian origin showed weak multifocal staining. One of 30 (3%) usual-type ENDO-CAs and 3/165 EM-CAs exhibited weak-moderate GATA3 immunoreactivity; all gastric-type cervical adenocarcinomas were negative. GATA3 is a highly sensitive and specific marker for mesonephric lesions in the lower genital tract; however, its utility in the upper genital tract may be more limited. In addition, GATA3 can aid in distinguishing lower genital mesonephric lesions from usual-type and gastric-type ENDO-CAs and uterine EM-CAs.

Key Words: mesonephric, cervix, GATA3, endocervical, endometrial, FATWO, immunohistochemistry, Müllerian, Wolffian

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GATA3, a member of the GATA family of transcription factors, is critical for embryogenesis, development, and cell differentiation in a variety of human tissues including breast, genitourinary tract, parathyroid, auditory tract, skin, and hematopoietic system (most notably for T-cell development and differentiation).[1–4] In mice, homozygous knockouts of *GATA3* are embryonic lethal,[5] and, in humans, *Gata3* haploinsufficiency causes a clinical syndrome called "hypoparathyroidism, deafness, and renal anomaly syndrome."[6,7] During embryogenesis, *GATA3* expression in the urogenital tract is regulated by *PAX2* and *PAX8*, with some redundancy.[8]

GATA3 has been proposed as a relatively sensitive and specific immunohistochemical (IHC) marker for urothelial, breast, and parathyroid carcinomas.[9–15] Relatively small studies looking at GATA3 expression in other tumor types revealed frequent positivity in paragangliomas, salivary gland tumors, ovarian Brenner tumors, and signet ring cell adenocarcinomas of the urinary bladder.[16,17] Less common expression has been documented in renal cell carcinomas, endometrial adenocarcinomas, and squamous cell carcinomas from the head/neck, lung, and cervix.[18–21] In a recent comprehensive analysis of GATA3 expression in human tumors, the spectrum of neoplasms commonly positive for GATA3 was expanded to include not only those previously mentioned, but also a subset of cutaneous basal cell carcinomas and other skin adnexal tumors, yolk sac tumor, choriocarcinoma, mesothelioma, pancreatic ductal adenocarcinoma, chromophobe renal cell carcinoma, oncocytoma, and the epithelial component of synovial

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sarcoma.[22] Despite the wide range of tumors demonstrating immunoreactivity for GATA3, it can still be a helpful diagnostic marker for specific differential diagnoses, especially when combined with other biomarkers.

Embryologically, the ejaculatory duct, seminal vesicle, vas deferens, and epididymis originate from the mesonephric/Wolffian ducts, and GATA3 is expressed in adult human Wolffian structures.[8,23,24] In formalin-fixed paraffin-embedded (FFPE) tissues, we have anecdotally observed GATA3 expression in the Wolffian-derived seminal vesicle and epididymis and, therefore, hypothesized that GATA3 would be expressed in benign mesonephric lesions and neoplasias in the female genital tract. Since the initiation of this study, 2 cases of mesonephric carcinosarcoma have been reported as GATA3 positive,[25] which supports our hypothesis. Therefore, the goal of this study was to evaluate GATA3 expression in a large cohort of mesonephric lesions, including mesonephric remnants (MRs), mesonephric hyperplasias (MHs), and neoplasms, including mesonephric carcinomas (MCAs) and female adnexal tumors of probable Wolffian origin (FATWOs). In addition, the use of GATA3 for distinguishing mesonephric lesions and Müllerian neoplasms with overlapping morphologies, such as usual-type endocervical and endometrial adenocarcinomas, was addressed.

MATERIALS AND METHODS

Selection of Cases and Histologic Diagnoses

A search of the Pathology archives at the Brigham and Women's Hospital, Boston, MA, the Massachusetts General Hospital, Boston, MA, and The Belfast Health and Social Care Trust, Belfast, Northern Ireland, United Kingdom, was performed to identify cases with "mesonephric" in the diagnosis. All cases with available material and histologically confirmed diagnoses were included in the study, which consisted of 59 FFPE samples, including 17 MRs (11 from the cervix and 6 from the fallopian tube), 15 cases of MH in the cervix, 21 MCAs (19 from cervix and 2 from the uterine corpus), and 6 FATWOs. Thirty "usual-type" endocervical adenocarcinomas (ENDO-CA), 9 "gastric-type" endocervical adenocarcinomas, and 165 endometrial adenocarcinomas (EM-CA) including 155 on a tissue microarray (152 endometrioid and 3 serous) and 10 whole-mount tissue sections (8 endometrioid and 2 serous) were also included for comparison.

Western Blot Analysis

To assess antibody specificity, a western blot for GATA3 protein expression was performed on 12 established cell lines, including breast (MCF7, T47D), bladder (T24), ovarian (Ovsaho, JHOS2, MCAS, EFO-27, ES2, TOV21G, TOV112D), endometrial (Hec-1A), and cervical (HeLa) cancer cell lines. Because no cell lines derived from MCA or mesonephric epithelia were available, cells from fresh human seminal vesicle obtained after radical prostatectomy were also included. Seminal vesicle epithelia were manually scraped with a scalpel blade from the luminal side of the seminal vesicle and placed in saline. Cells were lysed in RIPA buffer (Boston BioProducts, Ashland, MA) with Protease and Phosphatase Inhibitor cocktail (1:100 dilution, Thermo Scientific, Waltham, MA). Protein amounts were quantified using the Qubit protein assay (Life Technologies, Grand Island, NY), and 45 μg of total protein was loaded on a 4% to 12% NuPAGE Bis-Tris gel electrophoresis gel (Life Technologies). Proteins were transferred to a nitrocellulose membrane, which was then blotted with a mouse monoclonal anti-GATA antibody (1:500 dilution, Biocare Medical, Concord, CA) and a mouse monoclonal anti-GADPH (1:2000 dilution; Sigma, St Louis, MO); the latter served as a loading control. Anti-GATA3 antibody was incubated overnight at 4°C and anti-GADPH for 1 hour at room temperature. A horseradish peroxidase–linked anti-mouse IgG secondary antibody (1:2000 dilution; GE Healthcare, Bucks, UK) was used for detection (incubation for 1 h at room temperature). GADPH was developed using ECL 2 Western Blotting Substrate (Thermo Scientific), and GATA3 was developed using Supersignal West Femto Max Sensitivity Substrate (Thermo Scientific). Both were analyzed on the FlourChem HD2 imaging system (Alpha Innotech, San Leandro, CA).

Immunohistochemistry

GATA3 IHC was performed on all samples using standard techniques, the Envision Plus/Horseradish Peroxidase system (Dako, Carpinteria, CA), and a mouse monoclonal antibody to GATA3 (1:500 dilution, Biocare Medical, Concord, CA). Briefly, FFPE sections were incubated in hydrogen peroxide and absolute alcohol for 30 minutes to block endogenous peroxidase activity. Antigen retrieval was performed using pressure cooker pretreatment in 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 tissues were incubated using the Envision Plus secondary antibody for 30 minutes, followed by diaminobenzidine for 5 minutes. Appropriate positive (urothelial carcinoma) and negative (incubation with secondary antibody only) controls were stained in parallel. Only nuclear staining was considered positive, and GATA3 was semiquantitatively graded on the basis of intensity (weak, moderate, or strong), and extent (negative = < 1%, 1+ = 1% to 10%, 2+ = 11% to 50%, or 3+ = 51% to 100%) of staining.

Statistical Analysis

The sensitivity and specificity of GATA3 for cervical mesonephric lesions compared with ENDO-CA and EM-CA were calculated using an online clinical calculator (http://www.medcalc.org/calc/diagnostic_test.php).

RESULTS

Western Blot Analysis

Western blot analysis of fresh human mesonephric tissue (seminal vesicle) and various cancer cell lines showed that GATA3 protein expression was restricted to

mesonephric tissues and the breast and urothelial carcinoma cell lines (Fig. 1). None of the endocervical, endometrial, or ovarian cancer cell lines expressed detectable levels of GATA3 protein (Fig. 1). Moreover, we did not observe any other significant bands on the western blot. These findings support the specificity and sensitivity of the GATA3 antibody.

IHC Results for Non-neoplastic Tissues in the Lower Female Genital Tract

All non-neoplastic mesonephric cervical lesions demonstrated moderate to strong 3+ immunoreactivity with GATA3, including 11/11 (100%) MRs (Figs. 2A, B) and 15/15 (100%) MHs (Figs. 2C, D) (Table 1). Benign endocervical and endometrial epithelia were negative for GATA3, whereas squamous epithelium of the cervix demonstrated weak immunoreactivity in scattered epithelial cells (not shown).

IHC Results for Neoplasms in the Lower Female Genital Tract

Twenty of 21 (95%) MCAs were positive for GATA3, including 18/19 from the cervix (Fig. 3) and 2/2 from the uterine corpus (Fig. 4). However, both the extent and intensity of GATA3 positivity varied greatly (Table 1): 6/21(28%) MCAs showed 3+ staining (> 50% of tumor cells positive) with intensity varying from weak to strong, whereas 10/21 (48%) MCAs displayed 2+ staining (10% to 50% of tumor cells positive) also with variable intensity. In 4 cases (19%) staining was limited to < 10% of tumor cells (1+), being weak or moderate. Only 1 MCA was GATA3 negative. Notably, "poorly differentiated" areas, as evidenced by solid and/or spindled growth, present in 4 MCAs were uniformly negative for GATA3 in contrast to more well-differentiated areas of these tumors with tubular or papillary architecture (not shown).

Only 1 of 30 (3%) usual-type ENDO-CAs exhibited weak to moderate 2+ GATA3 immunoreactivity, whereas the remaining 29 cases (97%) were negative (Table 1). All 9 of the gastric-type adenocarcinomas were negative for GATA3 (Table 1). The vast majority of EM-CAs (162/165; 98%) were also negative for GATA3 including all 155 EM-CAs in the tissue microarray and 7 whole-mount tumor sections (6 endometrioid and 1 serous) (Table 1). Of the remaining 3 EM-CAs, 1 grade 2 endometrioid carcinoma demonstrated moderate 3+ GATA3 immunoreactivity, and 2 cases (1 grade 3 endometrioid and 1 serous) had weak 1+ staining. Overall, the sensitivity and specificity of GATA3 for benign and malignant mesonephric lesions in the cervix, when compared with endocervical and endometrial carcinomas, were 98% and 98%, respectively (Table 1).

IHC Results in the Upper Female Genital Tract

Five of 6 (83%) fallopian tube MRs were positive for GATA3 (Table 1). However, the staining pattern was more variable when compared with cervical MRs and hyperplasias: 4 cases stained 2+, ranging from moderate to strong in intensity, and 1 case was 1+ with weak intensity. Only 1/6 (17%) FATWOs demonstrated weak to moderate 2+ staining for GATA3 (Fig. 5), whereas the remaining 5 cases (83%) were negative (Table 1).

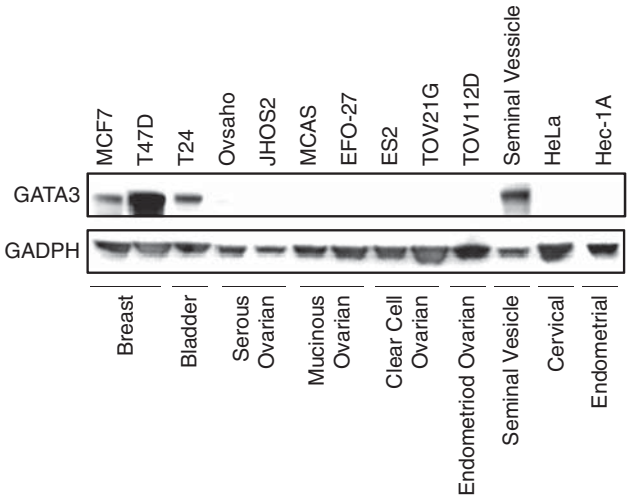

FIGURE 1. A western blot was performed to assess sensitivity and specificity for the GATA3 antibody. As expected, Wolffian tissue (seminal vesicle) and breast and bladder cancer cell lines were positive for expression of the GATA3 protein. In contrast, cervical, endometrial, and 4 ovarian (serous, mucinous, clear cell, and endometrioid) cancer cell lines were negative for GATA3 protein expression.

DISCUSSION

Mesonephric proliferations involving the cervix are relatively uncommon but may be confused with other neoplasms, particularly when located superficially or when florid in nature. Although the nuclear features of mesonephric lesions are typically bland and the mitotic activity low, the pseudoinfiltrative appearance deep in the cervical stroma or high up in the endocervical canal (Figs. 2A, C) may raise the possibility of an invasive ENDO-CA or EM-CA.[26–28] In contrast, some EM-CAs may invade the cervical stroma and undermine normal endocervical glands with little in the way of a stromal response, closely mimicking MRs or MHs.[29] Rarely, MCAs may arise as primary neoplasms in the wall of the uterine corpus (2 cases included in this study; Fig. 4) and may be a challenge to recognize.[30–33] In most instances, the diagnosis of a benign or malignant mesonephric lesion can be made on routine examination of hematoxylin and eosin–stained sections; however, in some cases, adjunctive IHC tests are helpful or even required. Previous studies have demonstrated that biomarkers can help support the diagnosis of MRs, hyperplasias, and carcinomas, but none has been shown to be entirely sensitive or specific. Such biomarkers include PAX8, PAX2, CD10, and calretinin.[30,31,34–40] The absence of hormone receptors (estrogen and progesterone receptors) by IHC can also be supportive of a mesonephric proliferation.[25,33,40–42]

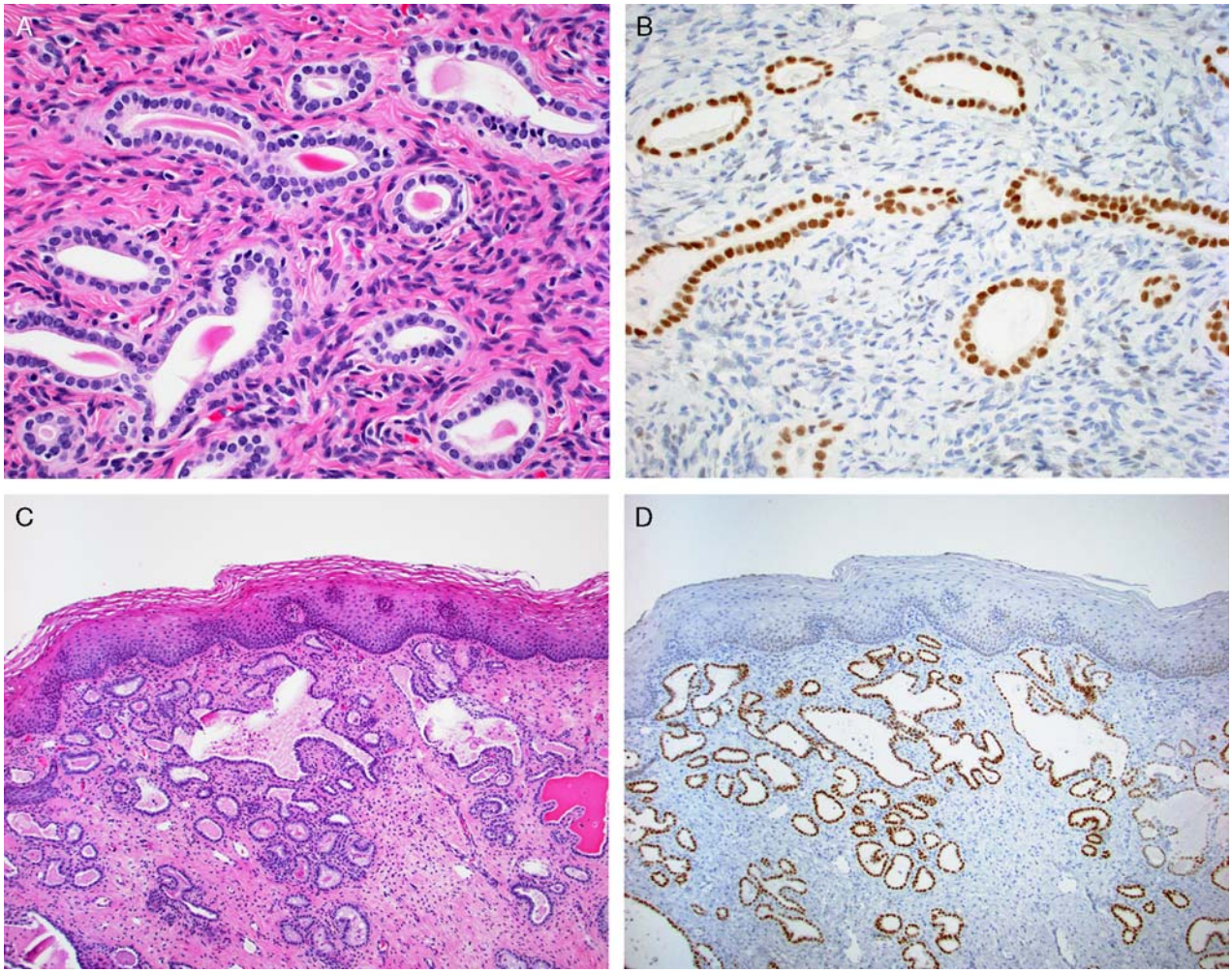

FIGURE 2. GATA3 demonstrated nuclear immunoreactivity in all MRs (A and B) and hyperplasias (C and D) in the cervix.

TABLE 1. GATA3 Staining Results in Cervical and Fallopian Tube MRs and Neoplasms, Compared with ENDO-CAs and EM-CAs

	No. Cases (%)	
	Positive*	**Negative**
Cervical MR (N = 11)	11 (100)	0
Cervical MH (N = 15)	15 (100)	0
MCA (N = 21)	20 (95)	1 (5)
FT MR (N = 6)	5 (83)	1 (17)
FATWO (N = 6)	1 (17)	5 (83)
ENDO-CA (N = 30)	1 (3)	29 (97)
Gastric-type ENDO-CA (N = 9)	0 (0)	9 (100)
EM-CA (N = 165)	3 (2)	162 (98)

Sensitivity of GATA3 for mesonephric lesions (including MR, MH, and MCA) versus nonmesonephric lesions (ENDO-CA, gastric-type cervical CA, EM-CA) is 97.87% (95% confidence interval: 88.66%-99.64%).

Specificity of GATA3 for mesonephric lesions (including MR, MH, and MCA) versus nonmesonephric lesions (ENDO-CA, gastric-type cervical CA, EM-CA) is 98.04% (95% confidence interval: 95.05%-99.45%).

*Positive staining defined as any intensity (weak/moderate/strong) and any extent (1+/2+/3+).

The family of PAX transcription factors has been well studied in the Müllerian tract, both developmentally and in neoplasms.[12,43–49] PAX8 is a well-known biomarker that is frequently used to support Müllerian, kidney, thyroid, and thymic neoplasia.[45,46,50] PAX8 is positive in the epididymis and seminal vesicle[46,51] and has been shown to stain MRs in the prostate[52] and cervix.[39,40] Therefore, PAX8 cannot be used to distinguish Müllerian from mesonephric lesions as benign and malignant mesonephric lesions are generally positive, as are endocervical, endometrial, and ovarian adenocarcinomas.[45] Expression of PAX2, another transcription factor required for development of the genitourinary tract (ie, Wolffian, Müllerian and renal structures),[53,54] is frequently lost in most upper and lower gynecologic tract adenocarcinomas, including serous, endometrioid, endocervical, and MCAs.[38,55,56] In contrast, MRs, MHs, and rare endocervical (usual and endometrioid types) and endometrial (endometrioid type) carcinomas have been shown to retain expression of PAX2.[38] This variable

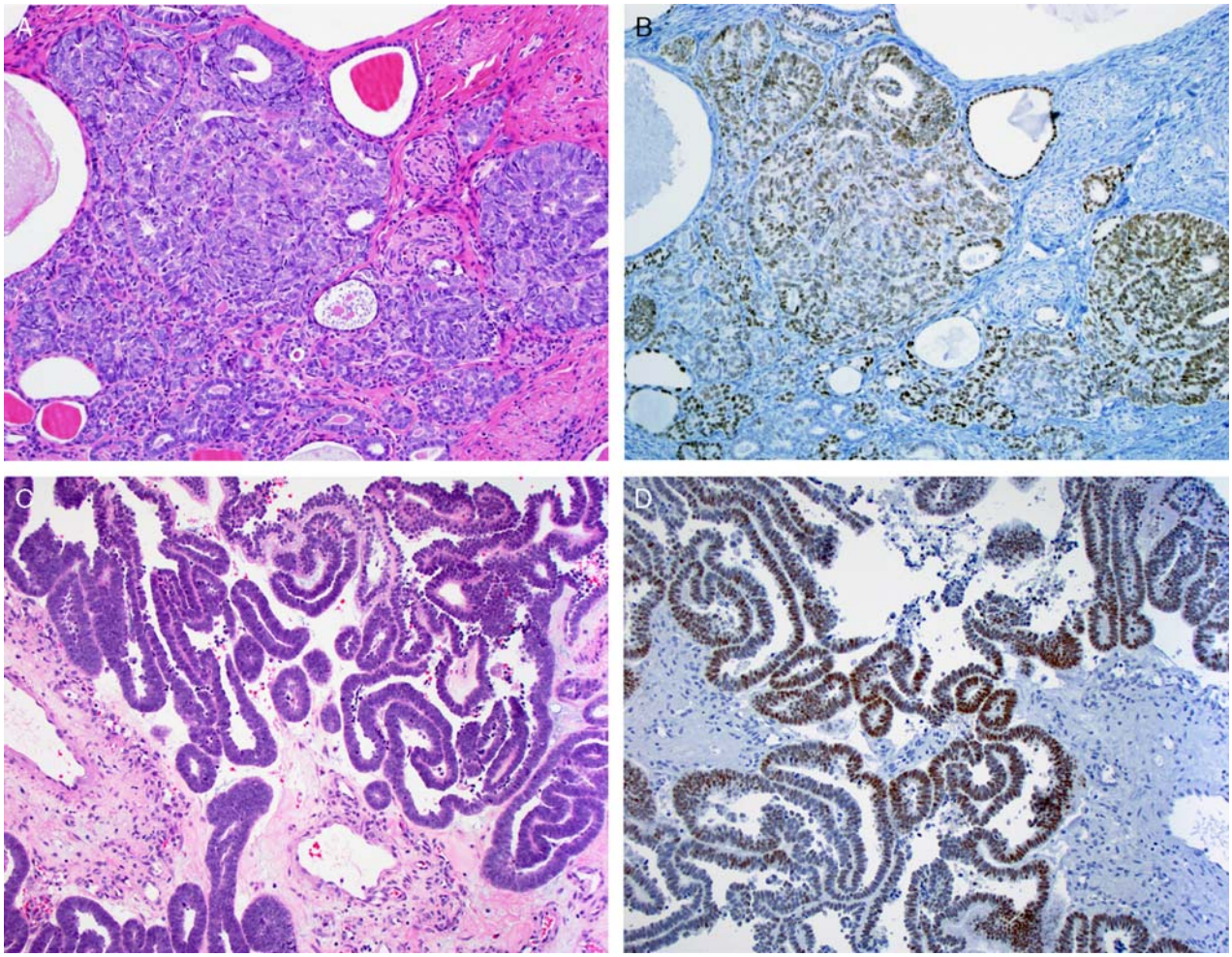

FIGURE 3. Two examples of MCA in the uterine cervix are strongly and diffusely positive for GATA3 (A–D). In this study only 1 of 19 MCAs located in the uterine cervix was negative for GATA3.

staining pattern for PAX2 suggests that it is a less than ideal biomarker for distinguishing mesonephric and Müllerian proliferations.

CD10 and calretinin are 2 of the most widely used biomarkers to support mesonephric differentiation.[30,31,34–37,39,57] Multiple studies have demonstrated that the majority of MRs, MHs, and MCAs are positive for CD10 (often with a luminal staining pattern), whereas calretinin is more typically positive in MCAs and less frequently in MRs and MHs. However, neither of these biomarkers is highly sensitive or specific for mesonephric differentiation, as both can be positive at least focally in a subset of endocervical (usual type) and endometrial (endometrioid type) carcinomas. Moreover, staining with these markers is often only focal in mesonephric lesions.

Studies have also looked at the use of p16 staining and human papillomavirus (HPV) detection to distinguish mesonephric lesions in the cervix from ENDO-CAs. In contrast to most conventional ENDO-CAs, which are typically diffusely positive for p16 and associated with high-risk HPV infection, benign and malignant mesonephric lesions demonstrate a variable staining pattern for p16 (usually negative or only focally positive) and are not associated with HPV infection.[39,40,58] Of note, the patchy p16 positivity in mesonephric lesions is similar to that seen in uterine endometrioid carcinomas.[59] Interestingly, it has been shown that there is a decrease in GATA3 mRNA and protein expression in immortalized HPV16-infected and HPV18-infected cervical cells when compared with noninfected epithelial and intraepithelial cervical lesions, suggesting a role for GATA3 deregulation in cervical carcinogenesis.[60] The absence of GATA3 by western blot analysis (Fig. 1) and IHC in the endocervical cell line and the whole-mount ENDO-CAs, respectively, in this study is consistent with these findings.

A potential pitfall for p16 includes some of the less common variants of cervical adenocarcinoma, such as minimal deviation adenocarcinoma (adenoma malignum) and gastric-type adenocarcinoma, which are HPV negative and either negative or only focally positive for p16.[58,61] Although the morphologic appearance of these

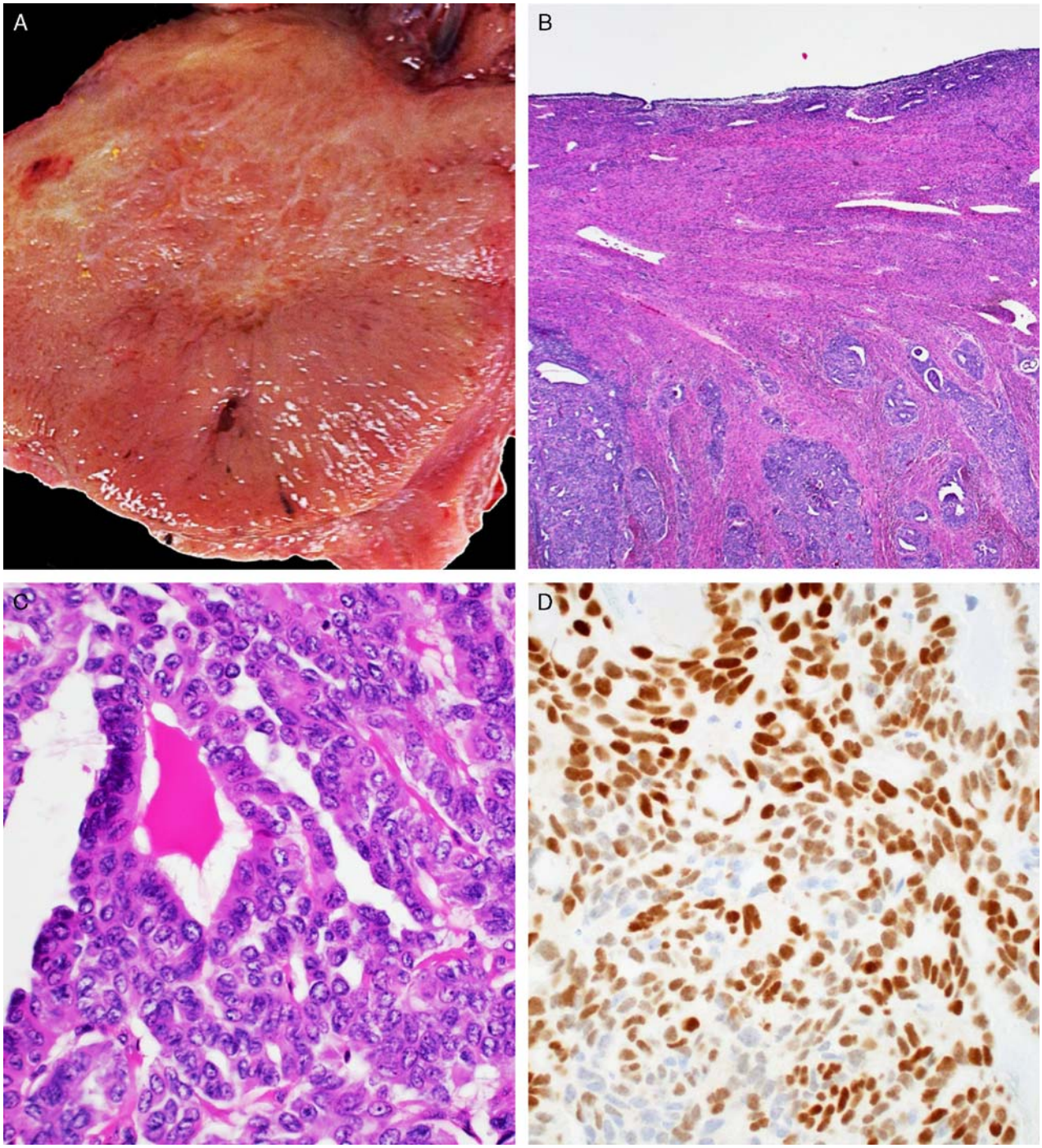

FIGURE 4. Two MCAs in the uterine corpus were evaluated, and both were immunoreactive with GATA3. A discrete lesion was grossly seen deep in the uterine wall (A); however, neoplastic epithelium infiltrated transmurally, but was absent in the overlying endometrium (B). At increased magnification the uterine MCA demonstrates classic cytologic features including uniform nuclei with open chromatin and nuclear grooves; bright intraluminal eosinophilic material is also present (C). In this case, GATA3 was expressed in >50% of tumor cells (3+), although the intensity varied from weak to strong (D).

unusual cervical adenocarcinoma variants is typically different from that of mesonephric proliferations, we have also shown that gastric-type cervical adenocarcinomas are negative for GATA3 (Table 1). Accordingly, GATA3 is a good biomarker when the differential diagnosis includes a p16-low to negative carcinoma and a mesonephric lesion. Additionally, as non-neoplastic endocervical glands were GATA3 negative in this study, this suggests that GATA3 may be useful when mesonephric lesions and pseudoneoplastic glandular lesions of the

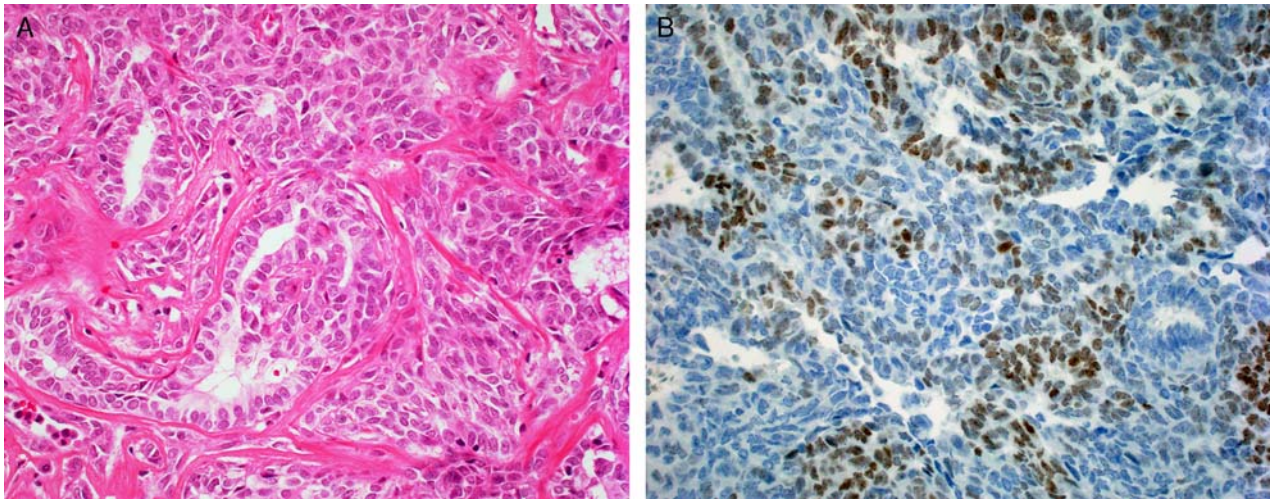

FIGURE 5. Only 1 of 6 FATWOs (A, H&E stain) demonstrated focal, weak to moderate GATA3 protein expression (B, by immunohistochemistry).

uterine cervix[62] are in the differential diagnosis; however, the latter were not investigated in this study.

Although this study demonstrates that GATA3 is both a sensitive and specific biomarker for benign and malignant mesonephric lesions of the cervix when the differential diagnosis includes ENDO-CA and EM-CA (Table 1), tumors in the cervix are not always primary to this site. Albeit infrequent, in some circumstances, one must also consider metastases to the cervix arising from extragenital locations such as the breast, bladder, and gastrointestinal tract, as well as other sites.[63–66] Such metastases may be located deep in the cervical wall or in parametrial soft tissue. When considering a diagnosis of MCA in this instance, relying solely on GATA3 expression for the diagnosis would be a pitfall, as most breast, urothelial, and some squamous carcinomas also express GATA3.[11,18,22] In such circumstances, morphology, clinical history, and additional immunostains may be helpful.

In contrast to mesonephric lesions in the cervix, GATA3 appears to be a much less reliable marker for mesonephric/Wolffian remnants and proliferations involving the adnexa (Table 1). Although most (5 of 6) MRs in the adnexa were positive for GATA3, the staining pattern was often only weak and focal. In addition, only 1 of 6 (17%) FATWOs demonstrated weak to moderate GATA3 immunoreactivity (Fig. 5). Notably, another study has shown that the immunoprofile of mesonephric lesions in the cervix and adnexa is not identical.[67] In this latter study, cervical MRs and carcinomas were typically positive for epithelial membrane antigen (EMA) and negative for inhibin, whereas the converse was true for the rete ovarii and FATWOs, although inhibin was typically only focally and weakly positive.[68] Additionally, although all of these structures arise from the mesonephric/Wolffian tubules during embryology, and are known to express PAX2 and PAX8,[51] some studies have shown a difference in gene expression in the cranial, anterior, and caudal portions of the mesonephric tubules, which ultimately become the testicular efferent ductules (cranial), the epididymis and vas deferens (anterior), and the seminal vesicles (caudal), respectively[69] (http://www.gudmap.org). In a mouse model, *Pkd1*, a gene encoding polycystin1 (PC1), which is important for the structural integrity of many tissues and organs, is required for normal development of the reproductive tract.[70] Interestingly, in the absence of *Pkd1* there was abnormal development of the efferent ductules (cranial mesonephros) and the epididymis (anterior mesonephros), but the seminal vesicles and ejaculatory ducts (caudal mesonephros) were not affected. On the basis of these findings, one could hypothesize that with mesonephric duct regression, in the absence of Müllerian inhibiting substance, some phenotypic differences might be seen in the "cranial" remnants along the adnexa and more "caudal" remnants along the lateral wall of the uterus/cervix (parametrial). Nevertheless, such findings would require additional studies.

In summary, this study demonstrates that GATA3 is a highly sensitive and specific biomarker for cervical mesonephric lesions, particularly when the differential diagnoses include ENDO-CA and EM-CA. In contrast, GATA3 appears to be a less reliable marker for mesonephric/Wolffian lesions around the adnexa, as most are either weakly positive or negative, possibly secondary to embryologic differences.

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