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The Potential Diagnostic Utility of TROP-2 in Thyroid Neoplasms

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Objectives: Human trophoblast cell-surface marker (TROP-2) has been reported to be overexpressed in various human carcinomas (CAs) and suggested to be a prognostic marker for some CAs. The diagnostic utility of TROP-2 in CAs has not been explored.

Methods: Immunohistochemical evaluation of TROP-2 expression on tissue microarray sections of 136 thyroid neoplasms, surgical specimens of 61 atypical thyroid follicular-patterned lesions [including 33 papillary thyroid carcinomas (PTCs), 17 atypical follicular neoplasms (AFNs), and 11 adenomatoid nodules with focal nuclear atypia (ANFNA)], and 20 benign thyroid lesions, as well as 10 cytology specimens of PTCs was performed. For comparison, immunoassay for Hector Battifora mesothelial-1 (HBME-1), galectin-3, and cytokeratin 19 was performed on the 61 atypical thyroid follicular-patterned lesions.

Results: Strong membranous staining with TROP-2 was seen in 94% (33/35) of classic PTCs and 81% (30/37) of confirmed follicular variant PTCs on tissue microarray and routine surgical sections, as well as 100% (10/10) of PTCs on cytology specimens; it was not observed in follicular adenomas (n = 51) or CAs (n = 37), AFNs or ANFNA (n = 28), benign (n = 20) or normal (n = 15) thyroid tissue. In contrast, the expression of HBME-1 and galectin-3 was identified in 100% (33/33) of surgical cases of PTCs and in 57% (16/28) and 50% (14/28) of AFNs and ANFNA, respectively.

Conclusions: Our findings demonstrate that a membranous TROP-2 staining pattern is highly specific for PTC, which may serve as a potential diagnostic marker aiding in the accurate classification of morphologically equivocal thyroid follicular-patterned lesions.

Received for publication November 6, 2015; accepted December 18, 2015.

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This study was partly presented at the USCAP Annual Meetings in 2013 and 2014.

The authors declare no conflict of interest.

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Key Words: TROP-2, thyroid neoplasms, normal thyroid

(*Appl Immunohistochem Mol Morphol* 2017;25:525–533)

The tumor-associated calcium signal transducer (TACSTD) gene family is comprised of 2 highly conserved and closely related genes, *TACSTD1* and *TACSTD2*, encoding epithelial cell adhesion molecule (TROP-1, also known as EpCAM) and TROP-2, respectively.[1,2] TROP-2, a 35 kDa, 323 amino acid, type 1 transmembranous glycoprotein, was originally identified in human trophoblast and choriocarcinoma cell lines[3–5] and subsequently reported to be overexpressed in a variety of human carcinomas (CAs), including colorectal, gastric and pancreatic CAs, squamous cell CAs of the oral cavity, non–small cell CAs of the lung, endometrial and ovarian malignancies, and only rarely in normal tissues.[4,6–11] Human transforming growth factor-β1–dependent epidermal Langerhans cells are also reported to express TROP-2.[12] TROP-2 overexpression in human CAs is associated with tumor aggressiveness and poor prognosis.[13–18] In recent years, TROP-2 has been actively studied as a prognostic marker and explored as an attractive immunotherapeutic target in human cancer treatment[19–29]; however, immunohistochemical (IHC) expression of TROP-2 in human neoplasms has not been evaluated for its diagnostic utilities.

In this study, we immunohistochemically evaluated TROP-2 (Cat. No. TROP-2[F-5]:sc-376181; Santa Cruz Biotechnology Inc., Dallas, TX) expression on tissue microarray (TMA) sections of 136 thyroid neoplasms and 15 normal thyroid tissues, surgical tissue sections of 61 atypical follicular-patterned lesions, and 20 benign thyroid lesions, as well as cytology cell block material of 10 papillary thyroid carcinomas (PTCs) and explored its potential diagnostic utility in accurate thyroid tumor classification, especially in thyroid follicular-patterned lesions.

MATERIALS AND METHODS

Construction of TMA Blocks

The study was approved by the Institutional Review Board at Geisinger Medical Center, Danville, Pennsylvania. A total of 136 thyroid neoplasms, including 48 PTCs [31 classic PTCs (cPTCs) and 17 follicular variant PTCs (FVPTCs)], 51 follicular adenomas, and 37 follicular CAs, as well as 15 cases of normal thyroid tissue

TABLE 1. IHC Staining Protocols and Antibody Information

Antibody	Vendor	Catalog #/Clonality	Dilution	Incubation Time/Temperature	AR Method/Time/Temperature/pH
HBME-1	Cell Marque Corporation	283M-18/HBME-1	Predilute	32 min/37°C	Cell conditioning 1*/32 min/95°C/8.5
TROP-2	Santa Cruz Biotechnology	Sc-376181/F5	1:50	32 min/36°C	Cell conditioning 1/56 min/100°C/8.5
Galectin-3	Cell Marque Corporation	255M-18/9C4	Predilute	24 min/37°C	Cell conditioning 1/32 min/95°C/8.5
CK19	Ventana Medical Systems Inc.	760-4281/A53-/A2.26	Predilute	20 min/37°C	Cell conditioning 1/8 min/95°C/8.5

*Cell conditioning 1, Ventana Medical Systems Inc.
AR indicates antigen retrieval; CK19, cytokeratin 19; HBME-1, Hector Battifora mesothelial-1; TROP-2, human trophoblast cell-surface marker.

dating from 2000 to 2010 were retrieved from the archives of the Department of Laboratory Medicine at Geisinger Medical Center. Multiple TMA blocks with 2 punches of 0.75 or 1.0 mm each for each case were constructed as previously described.[30,31]

Routine Surgical Specimens

Sixty-one consecutive routine surgical cases of atypical follicular-patterned lesions were identified and retrieved based on the following: (1) cases sent out for expert consultation; (2) cases requiring IHC studies; (3) cases reported as "atypical follicular neoplasm (AFN)" or "follicular adenoma with atypical features" or "adenomatoid nodules with focal nuclear atypia (ANFNA)." The diagnoses were rendered by expert consult (4 cases) and/or IHC analyses for cytokeratin 19 (CK19) (Cat. No. 760-4281; Ventana Medical Systems Inc., Tucson, AZ), Hector Battifora mesothelial-1 (HBME-1) (Cat. No. 283 M-18; Cell Marque Corporation, Rocklin, CA), and galectin-3 (Cat. No. 255 M-18; Cell Marque Corporation) in conjunction with the histomorphology. The AFNs are those follicular adenomas with focal atypical nuclear features suggestive but not diagnostic of PTC. All cases were reviewed by 2 authors (H.L. and F.L.). These cases comprised 33 PTCs, including 9 FVPTCs and 24 thyroid papillary microcarcinomas [miPTCs (4 classic patterned and 20 follicular patterned)], 17 AFNs, and 11 ANFNAs. In addition, 10 surgical specimens each of nodular hyperplasia and chronic lymphocytic thyroiditis were also retrieved.

Fine Needle Aspiration (FNA) Biopsy Specimens

Ten cases of PTC on FNA specimens with confirmatory diagnoses on follow-up surgical resections were also identified and retrieved, including 6 cPTCs, 3 combined cPTCs and FVPTCs, and 1 FVPTC. All cases had cell block preparations containing at least 3 groups of tumor cells with at least 10 tumor cells for each group.

IHC Assay

IHC evaluation of TROP-2 expression on TMAs of 4-μm formalin-fixed and paraffin-embedded sections of 136 thyroid neoplasms and 15 normal thyroid tissues, of routine surgical cases of 61 atypical follicular-patterned lesions and 20 benign thyroid lesions (10 cases each of nodular hyperplasia and chronic lymphocytic thyroiditis), and of cell blocks of 10 cytology FNA specimens was performed using the BenchMark ULTRA (Ventana Medical Systems Inc.) staining platform. The staining protocol was described previously,[32,33] and is summarized in Table 1, also including detailed antibody information. The validated PTC tissue was used as positive control and the normal thyroid tissue as negative control. Only membranous staining was considered positive. The staining intensity was graded as weak or strong. The distribution was recorded as negative (no stain or $<5\%$ of tumor cells stained), 1+ (5% to 25%), 2+ (26% to 50%), 3+ (51% to 75%), or 4+ ($>75\%$). In normal thyroid tissues, the staining pattern (membranous or cytoplasmic) was noted. For the 61 surgical cases of atypical follicular-patterned lesions, IHC evaluations for HBME-1, galectin-3, and CK19 were also performed. The staining protocols and detailed antibody information are summarized in Table 1. Two surgical pathologists (H.L. and F.L.) independently evaluated the immunostained slides.

RESULTS

TROP-2 Expression in Thyroid Neoplasms on TMA Sections

TROP-2 expression in 136 thyroid neoplasms is summarized in Table 2. Briefly, 90% (43/48) of PTCs, including 97% (30/31) of cPTCs and 76% (13/17) of FVPTCs, demonstrated a strong membranous staining pattern, with the majority being diffuse, as illustrated in Figures 1A and B. In contrast, none of the follicular adenomas or follicular CAs expressed TROP-2 in a membranous pattern. Focal weak cytoplasmic staining in 2/51 follicular adenomas and in 4/37 follicular CAs was observed, as illustrated in Figures 1C and D.

TABLE 2. TROP-2 Expression in 136 Thyroid Neoplasms

Diagnosis (N)	1+	2+	3+	4+	Total Positive Cases [n (%)]
cPTC (31)	4	4	12	10	30 (97)
FVPTC (17)	2	3	6	2	13 (76)
FA (51)	0*	0	0	0	0
FC (37)	0†	0†	0	0	0

*2/51 of follicular adenomas showed 1+ moderate to strong cytoplasmic staining.
†3/37 of follicular carcinomas showed 1+ moderate to strong cytoplasmic staining; 1/37 showed 2+ moderate to strong cytoplasmic staining.
cPTC indicates classic papillary thyroid carcinoma; FA, follicular adenoma; FC, follicular carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; n, number of cases; TROP-2, human trophoblast cell-surface marker.

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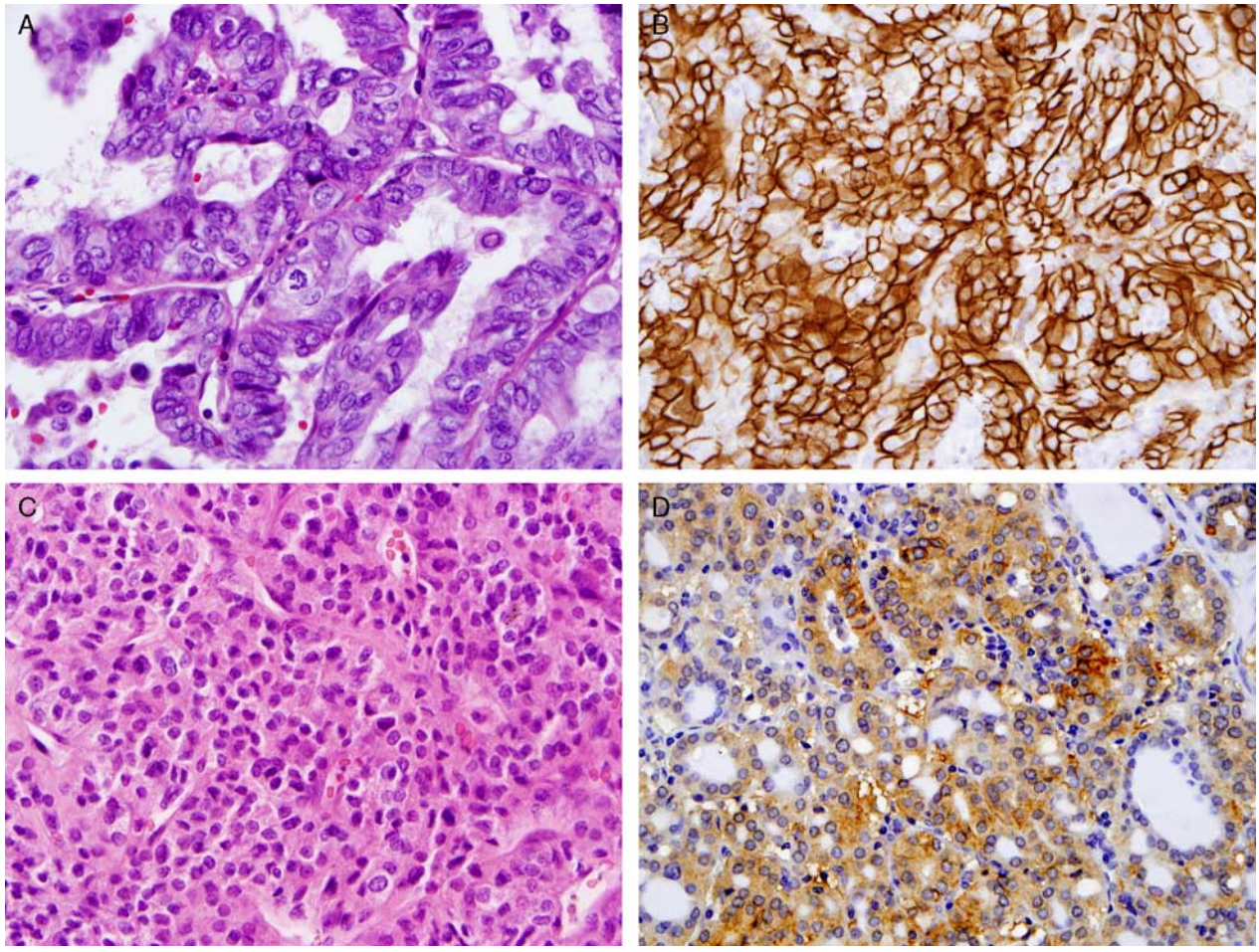

FIGURE 1. Human trophoblast cell-surface marker (TROP-2) staining patterns in thyroid neoplasms on tissue microarray cases. A, Papillary thyroid carcinoma [hematoxylin and eosin (H&E), ×40]. B, Strong membranous staining pattern. C, Thyroid follicular carcinoma (H&E, ×40). D, No TROP-2 membranous staining pattern was identified in follicular neoplasms.

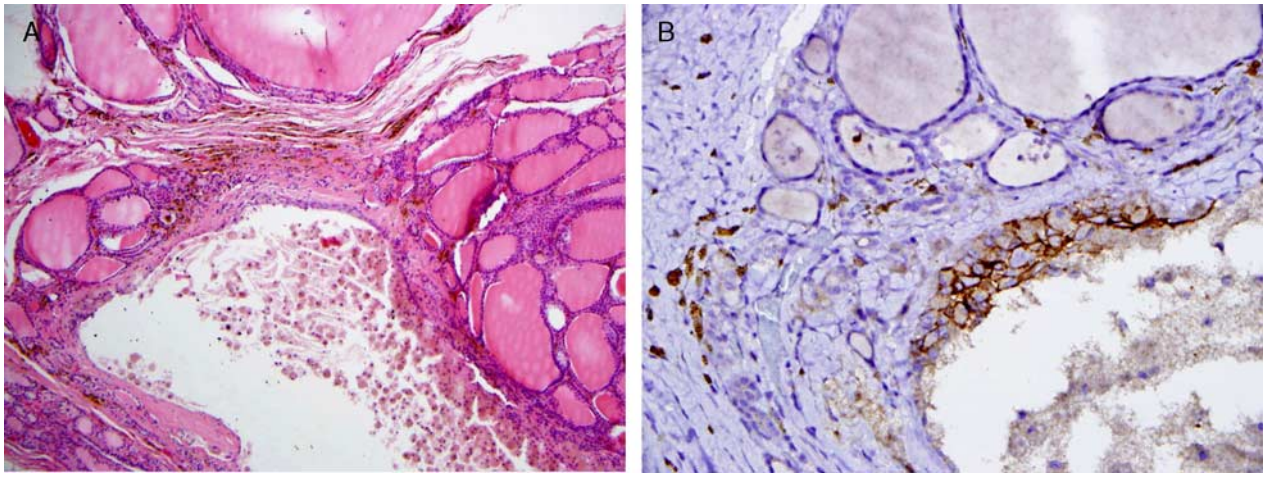

FIGURE 2. Human trophoblast cell-surface marker (TROP-2) staining pattern in benign thyroid lesions. A, A degenerative cyst in a case of chronic lymphocytic thyroiditis (hematoxylin and eosin, ×20). B, No TROP-2 expression was identified in 10 cases of nodular hyperplasia or 10 cases of chronic lymphocytic thyroiditis, except this cyst, showing focal weak to moderate membranous staining in the lining cells of the cyst.

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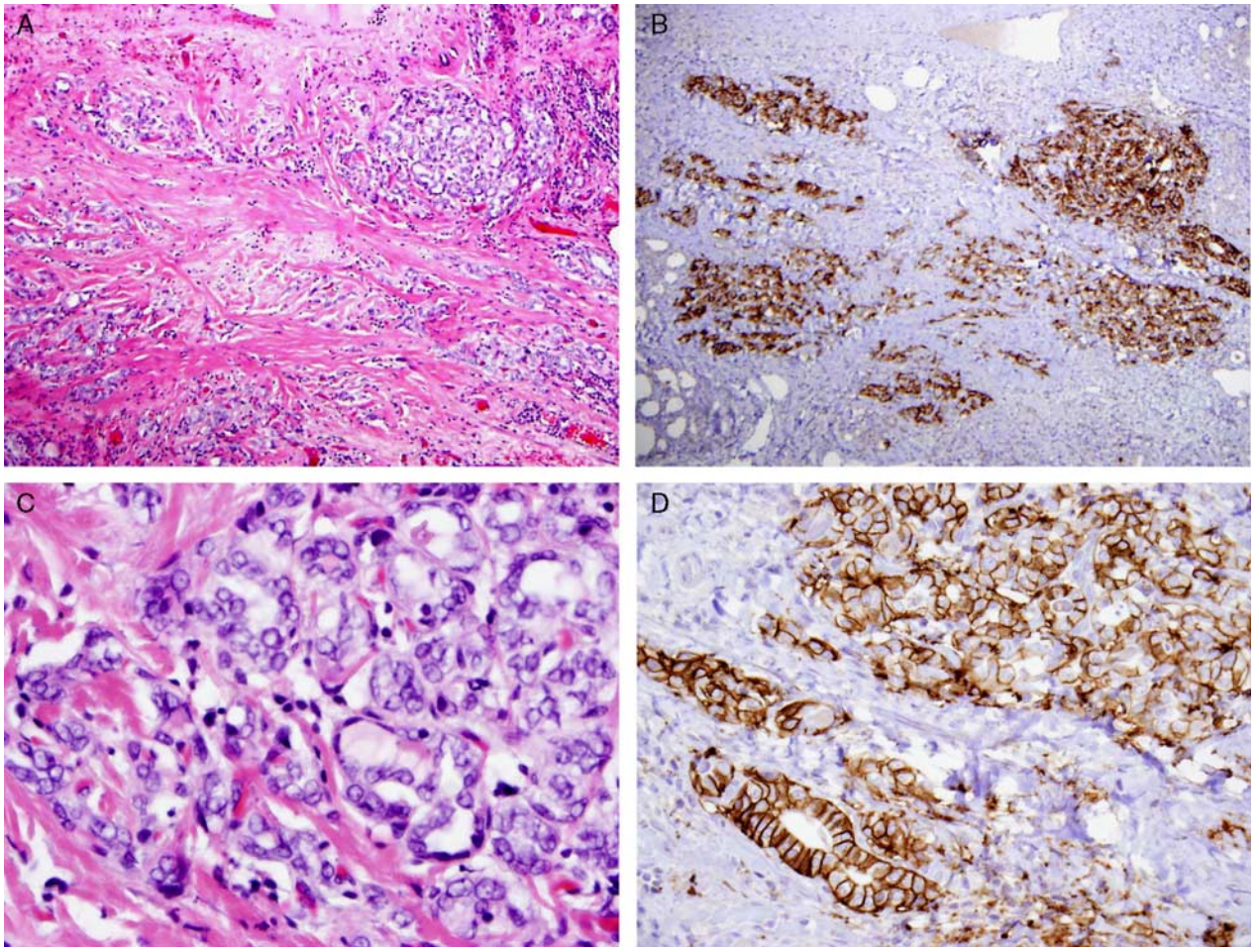

FIGURE 3. Human trophoblast cell-surface marker (TROP-2) staining pattern in 61 atypical follicular-patterned lesions. A, Thyroid papillary microcarcinoma (miPTC), follicular patterned [hematoxylin and eosin (H&E), ×10, low-power view]. B, TROP-2 highlights miPTC (low-power view). The nontumor thyroid tissue is nonreactive without any background stains. C, miPTC, follicular patterned (H&E, ×40, high-power view), showing nuclear clearing, grooving, and pseudoinclusions. D, miPTC, follicular patterned, diffuse TROP-2 membranous staining pattern (4+).

TROP-2 Expression in Normal and Benign Thyroid Tissues

TROP-2 membranous expression on TMA sections of normal thyroid tissues (15 cases) was not evident. The 10 surgical cases each of nodular hyperplasia and chronic lymphocytic thyroiditis showed no TROP-2 expression, except focal membranous staining in the lining cells of a degenerative cyst in one of the 10 cases of chronic lymphocytic thyroiditis, as illustrated in Figures 2A and B.

TROP-2 Expression in 61 Atypical Follicular Lesions on Routine Sections

Of the 61 atypical follicular-patterned lesions, 70% (23/33) of PTCs, including 33% (3/9) of FVPTCs, 75% (3/4) of classic patterned miPTCs, and 85% (17/20) of follicular patterned miPTCs, were positive for TROP-2, with a diffuse staining pattern (3+ or 4+) in 83% (19/23) of the positive cases, as illustrated in Figures 3A–D. No cases of AFNs or ANFNAs showed membranous TROP-2 expression. HBME-1 and galectin-3 expression was identified in 100% (33/33) of cases of PTC, 53% and 47% of AFNs, and 64% and 55% of ANFNAs, respectively. Examples are illustrated in Figures 4A–F. Expression of CK19 was identified in 67% of PTCs, 12% of AFNs, and 27% of ANFNAs. The staining results are summarized in Table 3. The TROP-2 expression pattern in 33 PTCs is detailed in Table 4.

TROP-2 Expression in FNA Biopsy Specimens

The immunostaining results for 10 cytology FNA biopsy specimens, including surgically proven diagnoses of 6 cPTCs, 1 FVPTC, and 3 combined cPTCs and FVPTCs, are summarized in Table 5. All cases (10/10) showed strong membranous staining for TROP-2, with diffuse staining in the majority of cases, as illustrated in Figures 5A–F.

DISCUSSION

Cytomorphology and histomorphology remain the gold standard in the classification of thyroid tumors. The diagnosis

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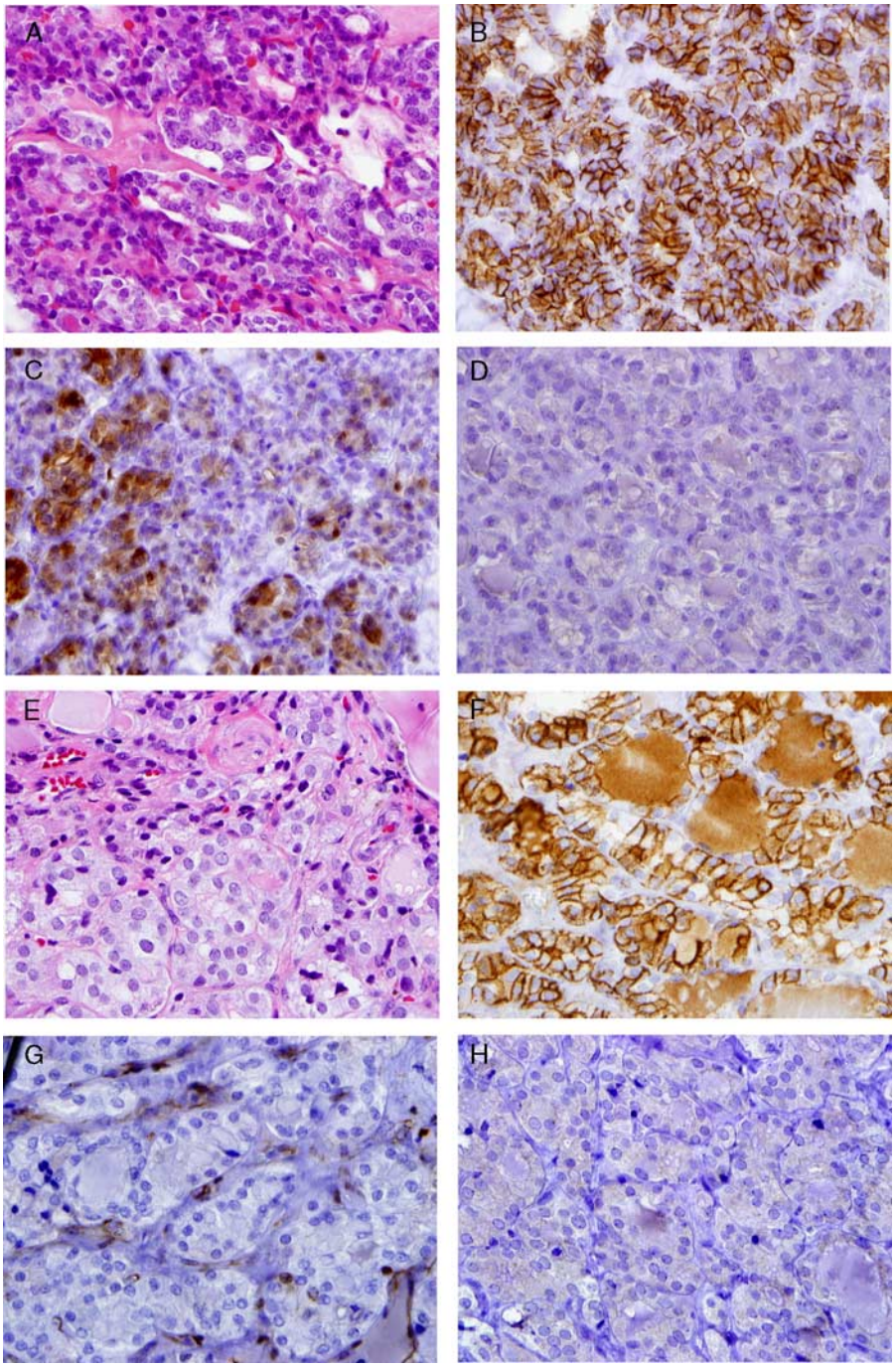

FIGURE 4. Examples of Hector Battifora mesothelial-1 (HBME-1) and galectin-3 staining pattern in atypical follicular neoplasms (AFN) and adenomatoid nodules with focal nuclear atypia (ANFNA). A, AFN [hematoxylin and eosin (H&E), ×20]. This is a 2.5 cm, well-circumscribed, partially encapsulated, solid nodule in a background of nodular hyperplasia, most consistent with a follicular adenoma; however, there are foci of atypical follicles showing nuclear enlargement, clearing, and grooving without definitive pseudoinclusions, as shown here. The atypical foci are scattered throughout the nodule although more prominent at the periphery. B, AFN, 3+ membranous and cytoplasmic staining for HBME-1. C, AFN, 2+ cytoplasmic staining for galectin-3. D, AFN, negative for human trophoblast cell-surface marker (TROP-2); also negative for cytokeratin 19 (CK19, not shown). E, ANFNA (H&E, ×40). This is a 0.8 cm well-circumscribed nodule (without capsule) in a background of multinodular goiter; there are patchy atypical follicular cells showing nuclear enlargement, clearing, and grooving without pseudoinclusions. F, ANFNA, 4+ membranous and cytoplasmic staining for HBME-1. G, ANFNA, negative for galectin-3. H, ANFNA, negative for TROP-2. In addition, CK19 is also negative (not shown). H&E indicates hematoxylin and eosin.

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TABLE 3. TROP-2 Expression in 61 Atypical Follicular-patterned Lesions

	n (%)			
Diagnosis (N)	**TROP-2**	**CK19**	**HBME-1**	**Galectin-3**
PTC (33)	23 (70)	22 (67)	33 (100)	33 (100)
AFN (17)	0	2 (12)	9 (53)	8 (47)
ANFNA (11)	0	3 (27)	7 (64)	6 (55)

AFN indicates atypical follicular neoplasm; ANFNA, adenomatoid nodules with focal nuclear atypia; CK19, cytokeratin 19; HBME-1, Hector Battifora mesothelial-1; n, number of cases; PTC, papillary thyroid carcinoma; TROP-2, human trophoblast cell-surface marker.

of cPTC is usually straightforward in both cytology and histology specimens; however, follicular-patterned lesions with equivocal cytologic and histologic features are frequently encountered. Those present a diagnostic challenge in the distinction of follicular neoplasm from PTC of the follicular variant. Interobserver and intraobserver disagreements in the diagnosis of follicular-patterned thyroid lesions are well documented, even among expert pathologists.[34–37] Ancillary studies, including IHC, are valuable tools aiding in the accurate classification of those morphologically equivocal lesions. Among the currently available immunomarkers, HBME-1, galectin-3, and CK19 are the most commonly used and are often recommended as a panel[38–47]; however, the specificity of those markers is poor, often decorating adenomas or adenomatoid nodules and showing more background stains, especially in areas of reactive changes, although diffuse expression has not been reported in benign reactive lesions in a majority of the studies. The lack of specificity of those immunomarkers limits their diagnostic utility, especially in limited samples such as cytology specimens.

Our initial findings of a distinct membranous staining pattern of TROP-2 in 90% (43/48) of PTCs on TMA sections and none of the thyroid follicular adenomas or CAs suggested the potential diagnostic utility of TROP-2 in the classification of thyroid neoplasms.[48] Further analysis of these data revealed that 97% (30/31) of cPTCs and 76% (13/17) of FVPTCs expressed TROP-2. To explore further, IHC evaluation of TROP-2 expression in 61 surgical cases of atypical follicular-patterned thyroid lesions was examined.[49] In addition, other commonly used biomarkers (HBME-1, galectin-3, and CK19) were compared. A strong membranous staining pattern was identified in 75% (3/4) of classic patterned miPTCs, 85% (17/20) of follicular patterned miPTCs, and 33% (3/9) of FVPTCs, but none (0/28) of the AFNs or ANFNAs. In contrast, 100% (33/33) of PTCs expressed HBME-1 and galectin-3; however, 57% (16/28) and 50% (14/28) of AFNs and/or ANFNAs were also positive, respectively, including diffuse staining (3+ or 4+) in 8/16 cases positive for HBME-1 and 2/14 cases positive for galectin-3; CK19 showed low sensitivity, with a positive rate of 67% for PTCs, whereas 12% of AFNs and 27% of ANFNAs were also reactive.

TABLE 4. TROP-2 Expression Pattern in 33 Papillary Thyroid Carcinomas

	TROP-2				
Diagnosis (N)	**1+**	**2+**	**3+**	**4+**	**Positive [n/N (%)**
FVPTC (9)	0	0	0	3	3/9 (33)
cmiPTC (4)	0	0	0	3	3/4 (75)
fvmiPTC (20)	0	4	1	12	17/20 (85)

cmiPTC indicates classic patterned papillary thyroid microcarcinoma; fvmiPTC, follicular patterned papillary thyroid microcarcinoma; FVPTC, follicular variant papillary thyroid carcinoma; n, number of cases; TROP-2, human trophoblast cell-surface marker.

The overall sensitivity of TROP-2 is 94% for cPTC and 81% for confirmed FVPTC. The group of 9 cases of FVPTC in the 61 surgical cases of atypical follicular-patterned thyroid lesions was excluded from this calculation due to the diagnostic uncertainty of several cases in this group. Comparing the overall sensitivity of TROP-2 in PTC on TMA sections (43/48, 90%) with that on surgical tissue sections (23/33, 70%), the higher sensitivity in the former may be attributed to the fact that these cases were larger in size (suitable for TMA construction) and had a cPTC morphology in a majority (31 cPTCs with a TROP-2-positive rate of 97%, 17 FVPTCs with a TROP-2-positive rate of 76%), whereas the PTC cases (n = 33) on surgical tissue sections were either miPTC (n = 24) or FVPTC (≥ 1 cm, n = 9) with equivocal histomorphology. The latter (9 cases of FVPTC, with the lowest TROP-2 sensitivity of 33%) included 4 cases showing the most equivocal histomorphology (sent out for expert consult), and therefore many of those may be controversial diagnoses. This group was excluded from the calculation for overall sensitivity. Among the 24 cases of miPTC, 3/4 (75%) classic patterned miPTCs and 17/20 (85%) follicular patterned miPTCs were TROP-2 positive. The sensitivities were similar when comparing FVPTC on TMA or routine surgical cases, except the 9 controversial cases of FVPTC on surgical specimens previously discussed. The follicular patterned miPTCs usually have more classic nuclear features of PTC and thus show a slightly higher sensitivity of 85%, as compared with that of FVPTC in general. Our study documented well that TROP-2 decorates cPTCs, with an overall sensitivity of 94% on TMA and surgical routine sections, although this finding has no practical value, as we all know that the diagnosis of cPTC needs no ancillary studies. The most meaningful findings of this study rest on the fact that TROP-2 expression in PTC of follicular variant (including cases with equivocal histomorphology) reaches a rate of 81% overall, including 76% (13/17) for FVPTC in TMA cases and 85% (17/20) for follicular patterned miPTCs on routine surgical sections; in contrast, none of the follicular adenomas, follicular CAs, other follicular-patterned lesions with atypical features, benign thyroid lesions, and normal thyroid tissues expressed TROP-2. The extremely high specificity and reasonable sensitivity of TROP-2 for PTC (including FVPTC) make TROP-2 an attractive immunomarker for the classification of thyroid tumors, especially for follicular-patterned lesions with equivocal histomorphology. Compared with the

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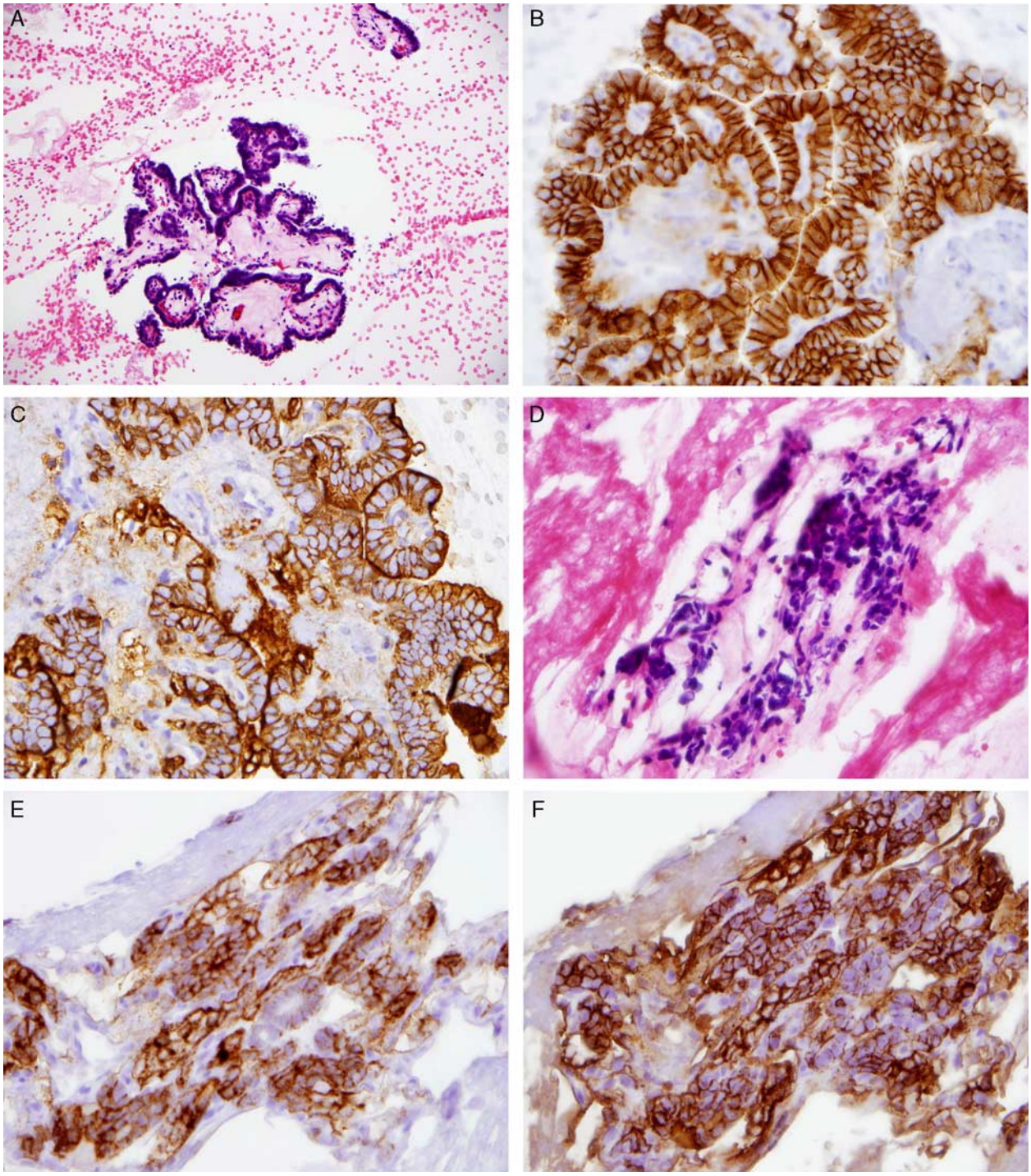

FIGURE 5. Human trophoblast cell-surface marker (TROP-2) immunostaining pattern in 10 cytology cases. A, Classic thyroid papillary carcinoma (cPTC), cell block preparation shows papillary clusters of tumor cells [hematoxylin and eosin (H&E), ×40). B, cPTC, diffuse, strong membranous staining pattern for TROP-2. C, cPTC, strong membranous and cytoplasmic staining for Hector Battifora mesothelial-1 (HBME-1). D, Follicular variant thyroid papillary carcinoma (FVPTC), cell block preparation shows clusters of tumor cells (H&E, ×40). E, FVPTC, diffuse, strong membranous staining pattern for TROP-2. F, FVPTC, strong membranous and cytoplasmic staining for HBME-1.

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TABLE 5. Immunostaining Pattern of Fine Needle Aspiration Cases

Case #	Surgical Diagnosis	TROP-2	CK19	HBME-1	Galectin-3
1	cPTC	4+, S (M)	ND	4+, S (M+C)	ND
2	cPTC	4+, S (M)	ND	ND	ND
3	cPTC	2+, S (M)	2+, S (M+C)	4+, S (M+C)	4+, S (C)
4	cPTC	4+, S (M)	4+, S (M+C)	4+, S (M+C)	4+, S (C)
5	cPTC	4+, S (M)	4+, S (M+C)	4+, S (M+C)	3+, S (C)
6	cPTC	4+, S (M)	2+, S (M+C)	4+, S (M+C)	4+, S (C)
7	FVPTC	4+, S (M)	4+, S (M+C)	3+, S (M+C)	4+, S (C)
8	FVPTC, cPTC	2+, S (M)	ND	4+, S (M+C)	ND
9	FVPTC, cPTC	4+, S (M)	3+, S (M+C)	4+, S (M+C)	4+, S (C)
10	FVPTC, cPTC	4+, S (M)	ND	ND	ND

C indicates cytoplasmic staining; CK19, cytokeratin 19; cPTC, classic papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; HBME-1, Hector Battifora mesothelial-1; M, membranous staining; ND, no data; S, strong; TROP-2, human trophoblast cell-surface marker.

staining patterns of HBME-1, galectin-3, and CK19, TROP-2 appeared highly specific, showed no background stains, and decorated tiny microscopic foci of PTC, which can be easily appreciated on low-power view. The application of TROP-2 in cytology cell block material was also initiated in 10 cases of surgically proven PTCs; all were positive, showing a strong membranous staining pattern ranging from 2+ to 4+.

It is worth briefly discussing here that TROP-2 is not a thyroid-specific biomarker; it can be expressed in CAs from various organs. Our data[48] on detection of TROP-2 expression in 1098 nonthyroidal tumors on TMA sections demonstrated that TROP-2 expression was not identified in neuroendocrine tumors/CAs of the pancreas and lung (n = 78), testicular tumors (n = 103), gastric adenocarcinomas (n = 21), hepatocellular CAs (n = 18), invasive lobular CAs of the breast (n = 31), or gastrointestinal stromal tumors (n = 36). Expression of TROP-2 in lung adenocarcinomas, lung squamous cell CAs, breast ductal CAs, pancreatic adenocarcinomas, and gynecologic CAs was variable. Of the urothelial CAs, 100% (38/38) of noninvasive, low-grade papillary urothelial CAs revealed a diffuse (3+ or 4+) strong membranous staining pattern, whereas 58% (25/43) of invasive urothelial CAs showed mainly a focal (1+ or 2+) weak to moderate membranous and cytoplasmic staining pattern. Of the kidney tumors studied, 3% (2/82) of the clear-cell renal cell carcinomas (RCCs), 3% (1/30) of the chromophobe RCCs/oncocytomas, and 44% (8/18) of the papillary RCCs expressed TROP-2. These findings suggest that TROP-2 may play a potential role in differentiating clear-cell RCC from papillary RCC. In addition, TROP-2 may play a role in the progression of urothelial CA as there was a marked reduction of TROP-2 expression in invasive urothelial CA when compared with noninvasive urothelial CA.

These data demonstrate that, among the thyroid lesions, a TROP-2 membranous staining pattern is specific for thyroid PTC, both classic and follicular variants. A small IHC panel including TROP-2 and HBME-1 is recommended as an initial panel to assist in the accurate classification of thyroid follicular-patterned lesions with equivocal morphologic features of cPTC. Further studies in a larger series of cases including an adequate number of FNA samples are needed to validate the current findings.

ACKNOWLEDGMENTS

The authors thank Melissa Erb for her outstanding secretarial support, Tina Brosious and Erin Powell for construction of TMA blocks and cutting TMA sections, Angie Bitting for her assistance with immunostains, and Kathy Fenstermacher for editing this manuscript.

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