The Potential Diagnostic Utility of TROP-2 in Thyroid Neoplasms

Haiyan Liu, MD, Jianhui Shi, MD, PhD, and Fan Lin, MD, PhD

**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, immunohasay 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.

From the Department of Laboratory Medicine, Geisinger Medical Center, Danville, PA.

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

The authors declare no conflict of interest.

Reprints: Haiyan Liu, MD, Department of Laboratory Medicine, MC 19-20, Geisinger Medical Center, 100N. Academy Ave., Danville, PA 17822 (e-mail: hliu1@geisinger.edu).

Copyright © 2016 The Author(s). Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.
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.

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 97% (30/31) of cPTCs and 76% (13/17) of FVPTCs, demonstrated a strong membranous 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.
13/37 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.
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.
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
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.
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. 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; 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. 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. 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.

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 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

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

| 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)     |
| ANFN (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.

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

| Diagnosis (N) | 1+ | 2+ | 3+ | 4+ | Positive [n/N (%)] |
|--------------|----|----|----|----|-------------------|
| FVPTC (9)    | 0  | 0  | 3  | 3  | 3/9 (33)          |
| cmiPTC (4)   | 0  | 0  | 3  | 3  | 3/4 (75)          |
| fvmiPTC (20) | 0  | 4  | 12 | 17| 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.
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.
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 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 Biting for her assistance with immunostains, and Kathy Fenstermacher for editing this manuscript.

REFERENCES

1. Wenqi D, Li W, Shanshan C, et al. EpCAM is overexpressed in gastric cancer and its downregulation suppresses proliferation of gastric cancer. J Cancer Res Clin Oncol. 2009;135:1277–1285.
2. Cubas R, Li M, Chen C, et al. Trop2: a possible therapeutic target for late stage epithelial carcinomas. Biochim Biophys Acta. 2009;1796:309–314.
3. Ohmachi T, Tanaka F, Mimori K, et al. Clinical significance of TROP2 expression in colorectal cancer. Clin Cancer Res. 2006;12:3057–3063.
4. Fong D, Spizzo G, Gostner JM, et al. TROP2: a novel prognostic marker in squamous cell carcinoma of the oral cavity. Mod Pathol. 2008;21:186–191.
5. Lipinski M, Parks DR, Rouse RV, et al. Human trophoblast cell-surface antigens defined by monoclonal antibodies. Proct Natl Acad Sci U S A. 1981;78:5147–5150.
6. Alberti S, Miotti S, Stella M, et al. Biochemical characterization of Trop-2, a cell surface molecule expressed by human carcinomas: formal proof that the monoclonal antibodies T16 and MOv-16 recognize Trop-2. Hybridoma. 1992;11:539–545.
7. Sukhthankar M, Alberti S, Baek SJ. (-)-Epigallocatechin-3-gallate (EGCG) post-transcriptionally and post-translationally suppresses the cell proliferative protein TROP2 in human colorectal cancer cells. Anticancer Res. 2010;30:2497–2503.
8. Mühlmann G, Spizzo G, Gostner J, et al. TROP2 expression as prognostic marker for gastric carcinoma. J Clin Pathol. 2009;62:152–158.
9. Fong D, Moser P, Krammel C, et al. High expression of TROP2 correlates with poor prognosis in pancreatic cancer. Br J Cancer. 2008;99:1290–1295.
10. Pak MG, Shin DH, Lee CH, et al. Significance of EpCAM and TROP2 expression in non-small cell lung cancer. World J Surg Oncol. 2012;10:53.
11. Fornano M, Dell’Arciprete R, Stella M, et al. Cloning of the gene encoding Trop-2, a cell-surface glycoprotein expressed by human carcinomas. Int J Cancer. 1995;62:610–618.
12. Eisenwort G, Jurkin J, Yasmin N, et al. Identification of TROP2 (TACSTD2), an EpCAM-like molecule, as a specific marker for TGF-β1-dependent human epithelial Langerhans cells. J Invest Dermatol. 2011;131:2049–2057.
13. Stepan LP, Trueblood ES, Hale K, et al. Expression of Trop2 cell surface glycoprotein in normal and tumor tissues: potential implications as a cancer therapeutic target. *J Histochem Cytochem*. 2011;59:701–710.

14. Kobayashi H, Minami Y, Anami Y, et al. Expression of the GA733 gene family and its relationship to prognosis in pulmonary adenocarcinoma. *Virchows Arch*. 2010;457:69–76.

15. Ning S, Guo S, Xie J, et al. TROP2 correlates with microvessel density and poor prognosis in hilar cholangiocarcinoma. *J Gastrointest Surg*. 2013;17:360–368.

16. Bignotti E, Todeschini P, Calza S, et al. Trop-2 overexpression as an independent marker for poor overall survival in ovarian carcinoma patients. *Eur J Cancer*. 2010;46:944–953.

17. Kobel M, Kalloger SE, Boyd N, et al. Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. *PloS Med*. 2008;5:e232.

18. Fang YJ, Lu ZH, Wang GQ, et al. Elevated expressions of MMP7, TROP2, and survivin are associated with survival, disease recurrence, and liver metastasis of colon cancer. *Int J Colorectal Dis*. 2009;24:875–884.

19. Wang J, Day R, Dong Y, et al. Identification of Trop-2 as an oncogene and an attractive therapeutic target in colon cancers. *Mol Cancer Ther*. 2008;7:280–285.

20. Cubas R, Zhang S, Li M, et al. Chimeric Trop2 virus-like particles: a potential immunotherapeutic approach against pancreatic cancer. *J Immunother*. 2011;34:251–263.

21. Bignotti E, Zanotti L, Calza S, et al. Trop-2 protein overexpression is an independent marker for predicting disease recurrence in endometrial endometrioid carcinoma. *BMC Clin Pathol*. 2012;12:22.

22. Nakashima K, Shimada H, Ochiai T, et al. Serological identification of TROP2 by recombinant cDNA expression cloning using sera of patients with esophageal squamous cell carcinoma. *Int J Cancer*. 2004;112:1029–1035.

23. Wu H, Xu H, Zhang S, et al. Potential therapeutic target and independent prognostic marker of TROP2 in laryngeal squamous cell carcinoma. *Head Neck*. 2013;35:1373–1378.

24. Wu M, Liu L, Chan C. Identification of novel targets for breast cancer by exploring gene switches on a genome scale. *BMC Genomics*. 2011;12:547.

25. Bignotti E, Ravaggi A, Romani C, et al. Trop-2 overexpression in poorly differentiated endometrial endometrioid carcinoma: implications for immunotherapy with hRS7, a humanized anti-trop-2 monoclonal antibody. *Int J Gynecol Cancer*. 2011;21:1613–1621.

26. Varughese J, Cocco E, Bellone S, et al. Cervical carcinomas overexpress human trophoblast cell-surface marker (Trop-2) and are highly sensitive to immunotherapy with hRS7, a humanized monoclonal anti-Trop-2 antibody. *Am J Obstet Gynecol*. 2011;205:567.e1–567.e7.

27. Varughese J, Cocco E, Bellone S, et al. High-grade, chemotherapy-resistant primary ovarian carcinoma cells line express human trophoblast cell-surface marker (Trop-2) and are highly sensitive to immunotherapy with hRS7, a humanized monoclonal anti-Trop-2 antibody. *Gynecol Oncol*. 2011;122:171–177.

28. Varughese J, Cocco E, Bellone S, et al. Uterine serous papillary carcinomas overexpress human trophoblast-cell-surface marker (Trop-2) and are highly sensitive to immunotherapy with hRS7, a humanized anti-Trop-2 monoclonal antibody. *Cancer*. 2011;117:3163–3172.

29. Mangino G, Grazia Capri M, Barnaba V, et al. Presentation of native TROP2 tumor antigens to human cytotoxic T lymphocytes by engineered antigen-presenting cells. *Int J Cancer*. 2002;101:353–359.

30. Lin F, Zhang PL, Yang XJ, et al. Human kidney injury molecule-1 (hKIM-1): a useful immunohistochemical marker for diagnosing renal cell carcinoma and ovarian clear cell carcinoma. *Am J Surg Pathol*. 2007;31:371–381.

31. Wilkerson ML, Powell E. Tissue microarray. In: Lin F, Prichard J, Liu H, et al. eds. *Handbook of Practical Immunohistochemistry—Frequently Asked Questions*, New York, NY: Springer; 2011:45–54.

32. Lin F, Shi J, Liu H, et al. Diagnostic utility of S100P and von Hippel-Lindau gene product (pVHL) in pancreatic adenocarcinoma—with implication of their roles in early tumorigenesis. *Am J Surg Pathol*. 2008;32:78–91.

33. Lin F, Shi J, Liu H, et al. Immunohistochemical detection of the von Hippel-Lindau gene product (pVHL) in human tissues and tumors: a diagnostic marker for metastatic renal cell carcinoma and clear cell carcinoma of the ovary and uterus. *Am J Clin Pathol*. 2008;129:592–605.

34. Hirokawa M, Carney JA, Goellner JR, et al. Observer variation of encapsulated follicular lesions of the thyroid gland. *Am J Surg Pathol*. 2002;26:1508–1514.

35. Franc B, de la Salomnie P, Lange F, et al. Interobserver and intraobserver reproducibility in the histopathology of follicular thyroid carcinoma. *Hum Pathol*. 2003;34:1092–1100.

36. Lloyd RV, Erickson LA, Casey MB, et al. Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma. *Am J Surg Pathol*. 2004;28:1336–1340.

37. Elsheikh TM, Asa SL, Chan JK, et al. Interobserver and intraobserver variation among experts in the diagnosis of thyroid follicular lesions with borderline nuclear features of papillary carcinoma. *Am J Clin Pathol*. 2008;130:736–744.

38. de Matos LL, Del Giglio AB, et al. Expression of CK-19, galectin-3 and HBME-1 in the differentiation of thyroid lesions: systematic review and diagnostic meta-analysis. *Diagn Pathol*. 2012;7:97.

39. Nakamura N, Erickson LA, Jin L, et al. Immunohistochemical separation of follicular variant of papillary thyroid carcinoma from follicular adenoma. *Endocr Pathol*. 2006;17:213–223.

40. Paunovic I, Ilic T, Havelka M, et al. Combined immunohistochemistry for thyroid peroxidase, galectin-3, CK19 and HBME-1 in differential diagnosis of thyroid tumors. *APMIS*. 2012;120:368–379.

41. Barut F, Onak Kandemir N, Bektas S, et al. Universal markers of thyroid malignancies: galectin-3, HBME-1, and cytokeratin-19. *Endocr Pathol*. 2010;21:80–89.

42. Rossi ED, Raffaelli M, Mue L, et al. Simultaneous immunohistochemical expression of HBME-1 and galectin-3 differentiates papillary carcinomas from hyperfunctioning lesions of the thyroid. *Histopathology*. 2006;48:795–800.

43. Prasad ML, Pellegrina NS, Huang Y, et al. Galectin-3, fibronectin-1, CITED-1, HBME1 and cytokeratin-19 immunostaining is useful for the differential diagnosis of thyroid tumors. *Mod Pathol*. 2005;18:48–57.

44. de Matos PS, Ferreira AP, de Oliveira Facuri F, et al. Usefulness of HBME-1, cytokeratin 19 and galectin-3 immunostaining in the diagnosis of thyroid malignancies: galectin-3, HBME-1, and cytokeratin-19. *Endocr Pathol*. 2010;21:80–89.

45. Beesley MF, McLaren KM. Cytokeratin 19 and galectin-3 immunohistochemistry in the differential diagnosis of solitary thyroid nodules. *Histopathology*. 2002;41:236–243.

46. Scognamiglio T, Hynek E, Kao J, et al. Diagnostic usefulness of HBME1, galectin-3, CK19, and CITED1 and evaluation of their expression in encapsulated lesions with questionable features of papillary thyroid carcinoma. *Am J Clin Pathol*. 2006;126:700–708.

47. Liu H, Lin F. Application of immunohistochemistry in thyroid pathology. *Arch Pathol Lab Med*. 2015;139:67–82.

48. Liu H, Shi J, Lin F. TROP2 expression in various tumors—a potential diagnostic marker for papillary thyroid carcinoma [USCAP abstract 558]. *Mod Pathol*. 2013;26(suppl 2s):134A.

49. Liu H, Shi H, Prichard J, et al. TROP-2 is a potential novel immunomarker for identification of papillary thyroid carcinomas [USCAP abstract 627]. *Mod Pathol*. 2014;27(suppl 2s):15A.