Diagnostic Use of PAX8, CAIX, TTF-1, and TGB in Metastatic Renal Cell Carcinoma of the Thyroid

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Abstract: Clear-cell renal cell carcinoma (ccRCC) may present with metastatic lesions in patients with a concurrent undiagnosed primary or a remote history of ccRCC. The thyroid is not uncommonly involved by metastatic ccRCC, in which a metastasis could be misinterpreted as a clear-cell change in adenomatoid nodules, follicular adenomas, or parathyroid glands. PAX8 is a transcription factor expressed by thyroid and renal-lineage cells. No previous study has evaluated the diagnostic use of PAX8 and ccRCC marker carbonic anhydrase IX (CAIX) in this setting. Cases of metastatic ccRCC in the thyroid (n = 12), parathyroid glands and adenomas with clear-cell change (n = 6), papillary thyroid carcinoma (n = 6), thyroid follicular adenomas (n = 5), and adenomatoid nodules with clear-cell change (n = 5) were studied. Cases were assessed by standard immunohistochemistry for thyroid transcription factor-1 (TTF-1), thyroglobulin (TGB), PAX8, and CAIX. The extent and intensity of nuclear or cytoplasmic immunoexpression were assessed, with any labeling considered as a positive result. All metastatic ccRCCs were positive for PAX8 (moderate-to-strong, patchy-to-diffuse) and CAIX (strong, diffuse), and were negative for TTF-1 and TGB. All primary thyroid lesions labeled strongly and diffusely for TTF-1, TGB, and PAX8, and were negative for CAIX. Parathyroid tissues were negative for TTF-1, TGB, PAX8, and CAIX. An immunoprofile of "TTF1(−)/TGB(−)/CAIX(+)" was 100% sensitive and specific for metastatic ccRCC of the thyroid. The reverse profile "TTF1(+)/TGB(+)/CAIX(−)" supported a primary thyroid lesion. PAX8 was not useful in distinguishing metastatic ccRCC from thyroid lesions.

Key Words: renal cell carcinoma, thyroid metastases, PAX8, TTF1, CAIX

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Renal cell carcinoma accounts for approximately 3% of all adult malignancies,[13] and up to 30% of patients have metastatic disease at presentation.[16] Conventional or clear-cell renal cell carcinoma (ccRCC) comprises 75% of renal cell carcinomas,[6] and it is the most common type of tumor overall to metastasize in the thyroid in clinical series.[17] In the thyroid, metastatic ccRCC could be misinterpreted as a clear-cell change in adenomatoid nodules, follicular adenomas, or parathyroid glands.[10]

PAX8 is a transcription factor expressed by both thyroid and renal-lineage cells.[7,8,12,14,20] Carbonic anhydrase IX (CAIX) is a downstream effector of the hypoxia inducible pathway, which is expressed in RCC.[1,4,9] CAIX protein expression has been studied in a wide range of normal and neoplastic human tissues,[11] but its expression in thyroid has not been studied. No previous study has evaluated the diagnostic use of immunohistochemistry (IHC) for PAX8 and CAIX in the setting of metastatic ccRCC in the thyroid.

MATERIALS AND METHODS

Eighteen cases of metastatic ccRCC in the thyroid (Fig. 1A) were identified in our archives over a 25-year period (1985 to 2010), and paraffin-embedded blocks of tissues were available for 12 of those cases. Clinicopathologic data were collected, including patient sex, age, history of previously known renal malignancy, size, and location of metastatic disease, and concomitant additional thyroid pathology. In addition, cases of parathyroid glands and parathyroid adenomas with clear-cell change (Fig. 1B) (n = 6), papillary thyroid carcinoma (n = 6), adenomatoid nodules with clear-cell change (Fig. 1C) (n = 5), and thyroid follicular adenomas (Fig. 1D) (n = 5) were also identified and studied.

IHC studies for thyroid transcription factor-1 (TTF-1) (Cell Marque; Rocklin, CA), thyroglobulin (TGB) (Cell Marque; Rocklin, CA), PAX8 (Protein Tech Group; Chicago, IL), and CAIX (Novacastra) were carried out on 5-μm-thick formalin-fixed, paraffin-embedded sections using automated IHC stainers (Bond-Leica, Leica microsystems, Bannockburn, IL; or Ventana Benchmark XT, Ventana Medical Systems, Inc., Tucson, AZ). In brief, slides were deparaffinized and hydrated, followed by heat-induced antigen retrieval. Incubation with the primary antibody using optimal conditions was followed by development of immunostaining and counterstaining. The secondary antibody and detection was applied as per the instructions of the manufacturer. The negative controls used were sections of the study tissues with no primary antibody incubation. The antibody

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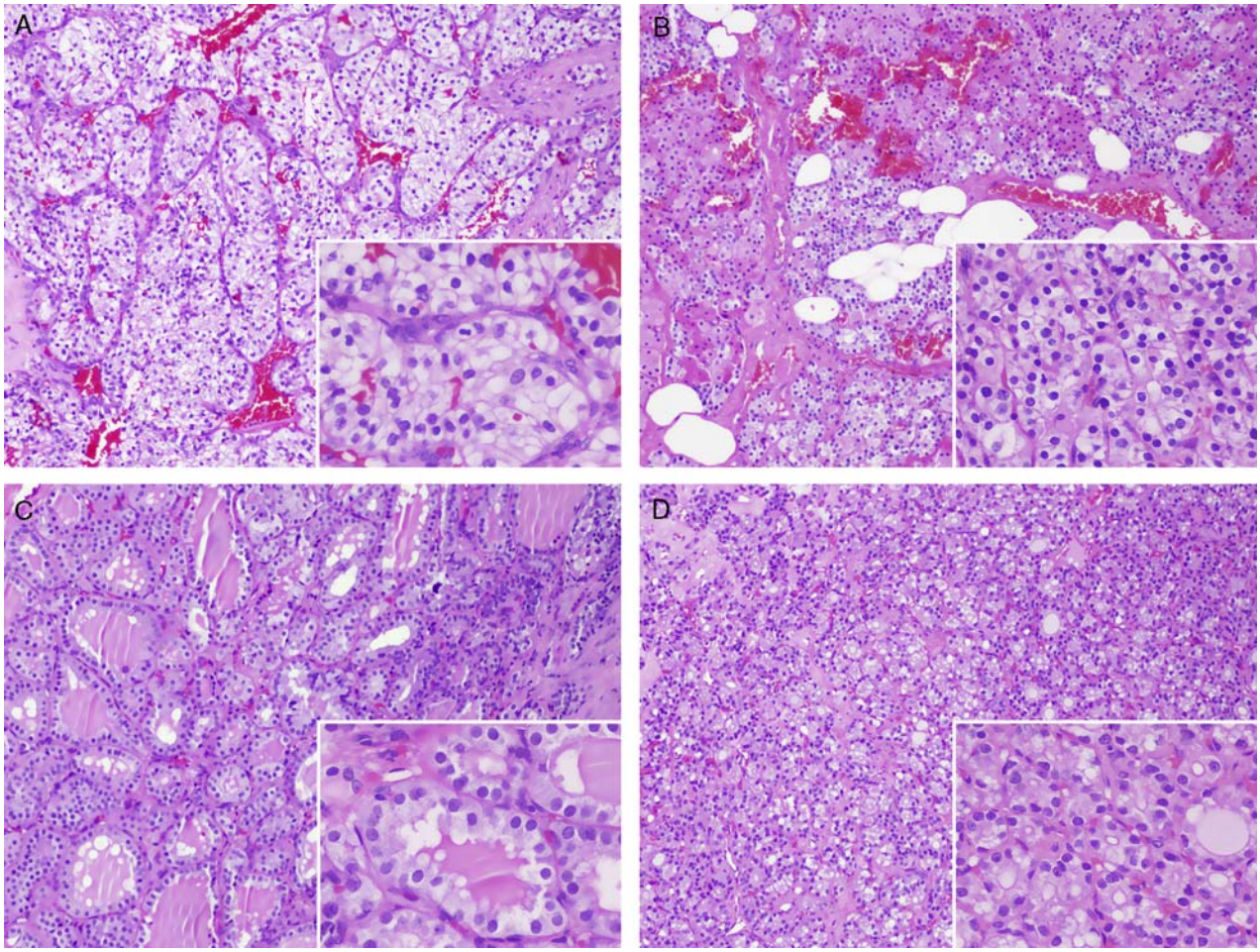

FIGURE 1. Histopathology of metastatic ccRCC of the thyroid, parathyroid gland adenoma, adenomatoid nodule, and follicular adenoma. Metastatic ccRCC in the thyroid (A) may be mistaken on routine histology for a parathyroid adenoma (B), adenomatoid nodule (C), or follicular adenoma (D) with clear-cell change. (Hematoxylin and eosin, $\times 10$ and $\times 40$).

specifications and incubation timings are described in Table 1. The extent and intensity of nuclear or cytoplasmic immunoexpression were assessed, with any labeling considered a positive result.

RESULTS

Metastatic ccRCC in the thyroid occurred in 7 men (58%) and 5 women (42%), at an average age of 68 years (range, 56 to 82 y). Three cases (25%) occurred in patients with no known history of renal cell carcinoma. Six cases consisted of solitary lesions, and 6 cases contained multiple foci of metastatic disease. One metastatic tumor was encapsulated. Two cases (17%) consisted of metastatic ccRCC involving adenomatoid nodules. One patient (8%) had a concomitant thyroid neoplasm (papillary thyroid carcinoma, uninvolved by ccRCC). One case was initially diagnosed as an adenomatoid nodule on preoperative fine needle aspiration (FNA), but a thyroidectomy was performed because of compressive symptoms. This patient had a remote history of renal cell carcinoma, unknown to the cytopathologist and endocrinologist at the time of FNA and surgery.

All metastatic ccRCCs were positive for PAX8 (moderate to strong, patchy or diffuse) and CAIX (strong,

TABLE 1. Antibody Specifications for TTF1, TGB, PAX8, and CAIX

Antibody	Source	Antibody Dilution	Epitope Retrieval
TTF1	Cell Marque; Rocklin, CA	Predilute	EDTA
TGB	Ventana; Tuscon, AZ	Predilute	EDTA
PAX8	Protein Tech Group; Chicago, IL	1:100	EDTA
CAIX	Leica; Bannockburn, IL	1:100	Citrate buffer

EDTA indicates ethylenediamineteraacetic acid.

TABLE 2. Immunostaining Pattern of Metastatic ccRCC, Primary Thyroid Lesions, and Parathyroid Lesions

		TTF-1	TGB	PAX8	CAIX
	No.	Percentage Positive	Percentage Positive	Percentage Positive	Percentage Positive
Metastatic clear cell RCC	12	0	0	100	100
Follicular adenoma	5	100	100	100	0
Adenomatoid nodule	5	100	100	100	0
Papillary thyroid carcinoma	6	100	100	100	0
Parathyroid gland and adenoma	6	0	0	0	0
Associated thyroid tissue	34	100	100	100	0

diffuse) and negative for TTF-1 and TGB (Table 2). All primary thyroid lesions (normal thyroid, papillary thyroid carcinomas, follicular adenomas, and adenomatoid nodules) labeled strongly and diffusely for TTF-1, TGB, and PAX8, and were negative for CAIX (Fig. 2). In 25% of the metastatic ccRCC cases, the adjacent normal thyroid parenchyma labeled more strongly and diffusely for PAX8 than did the metastatic lesions. The parathyroid gland tissue and adenomas were all negative for TTF-1, TBG, PAX8, and CAIX.

DISCUSSION

Renal cell carcinoma comprises approximately 3% of all adult malignancies[13] and is increasingly diagnosed as an incidental finding during routine radiologic imaging.[6,16] However, ccRCC may present with metastatic lesions in patients with concurrent clinically undiagnosed primary or a remote history of renal cancer; approximately one third of patients are diagnosed with metastatic disease at the time of presentation.[16]

Metastatic ccRCC most frequently involves the lung, bone, liver, and brain, but ccRCC also characteristically involves "unusual" sites of metastasis and can involve nearly any organ in the body such as the thyroid and pancreas.[16] Metastatic ccRCC is the most common metastasis to involve the thyroid gland, accounting for approximately 33% of all metastases in the thyroid in clinical material.[17] In the thyroid, metastatic ccRCC could be misinterpreted as a clear-cell change in adenomatoid nodules, follicular adenomas, or parathyroid gland adenomas. In one series of metastatic ccRCC in the thyroid gland, 36% of metastases were the initial manifestation of renal cell carcinoma in these patients.[10]

PAX8 is a transcription factor expressed by both thyroid and renal-lineage cells.[7,8,12,14,20] In the thyroid, its role has long been established as a transcriptional regulator by binding to the thyroperoxidase promoter.[7,14] Translocations involving chromosomes 2 and 3 create a fusion product of PAX8 and peroxisome proliferator-activated receptor-γ, which can be seen in follicular adenomas and carcinomas.[8] Immunolabeling for PAX8 is not routinely used in metastatic thyroid lesions, whereas immunolabeling for the transcription factor TTF1 is more commonly used.[8] The detection of PAX8 by IHC has been variable in thyroid neoplasms.[18,19] In contrast, PAX8 IHC labeling is frequently used to support the diagnosis of metastatic renal neoplasms.[2]

CAIX is a downstream effector of the von Hippel-Lindau hypoxia inducible pathway, which plays a major role in the pathogenesis of ccRCC, and CAIX can be detected by IHC in ccRCC and other renal neoplasms.[1,3,4,9] Its expression has been extensively and controversially investigated as an independent predictor of survival in ccRCC.[5,15] CAIX expression has been studied in a wide range of normal and neoplastic human tissues,[11] but its expression in thyroid has not been studied.

Although the diagnosis of metastatic ccRCC may be strongly suspected on the basis of histomorphology, IHC studies are often necessary to confirm the diagnosis, especially in patients with no known history of primary ccRCC. No previous study has evaluated the diagnostic use of PAX8 and CAIX in the setting of metastatic ccRCC in the thyroid. Here, we investigated the expression of PAX8, CAIX, TTF, and TGB in 12 cases of metastatic ccRCC in the thyroid and in parathyroid glands and adenomas with clear-cell change, papillary thyroid carcinomas, thyroid follicular adenomas, and adenomatoid nodules.

As expected, PAX8 is not a useful immunomarker in distinguishing metastatic ccRCC from primary thyroid lesions. Although PAX8 will be negative in parathyroid lesions, the marker still has limited use because positive staining does not further distinguish between thyroid and renal lesions. Additional markers that are specific for parathyroid, such as parathyroid hormone and chromogranin, can also be used in this setting. Histologic clues that support the diagnosis of parathyroid tissue include the presence of acidophilic oxyphil cells and admixed fibroadipose tissue (Fig. 1B).

An immunoprofile of "TTF1(−)/TGB(−)/CAIX(+)" was 100% sensitive and specific for metastatic ccRCC in the thyroid. The reverse profile "TTF1(+)/TGB(+)/CAIX(−)" supported a primary thyroid lesion. However, this study is limited to 12 cases, and these findings should be validated in a larger series.

Thyroid nodules are routinely sampled by FNA for diagnostic evaluation.[21] One of the cases of metastatic ccRCC included in this series was initially diagnosed as an adenomatoid nodule on preoperative FNA. A high index of suspicion must be maintained for metastatic ccRCC in the thyroid in an FNA containing cells with a

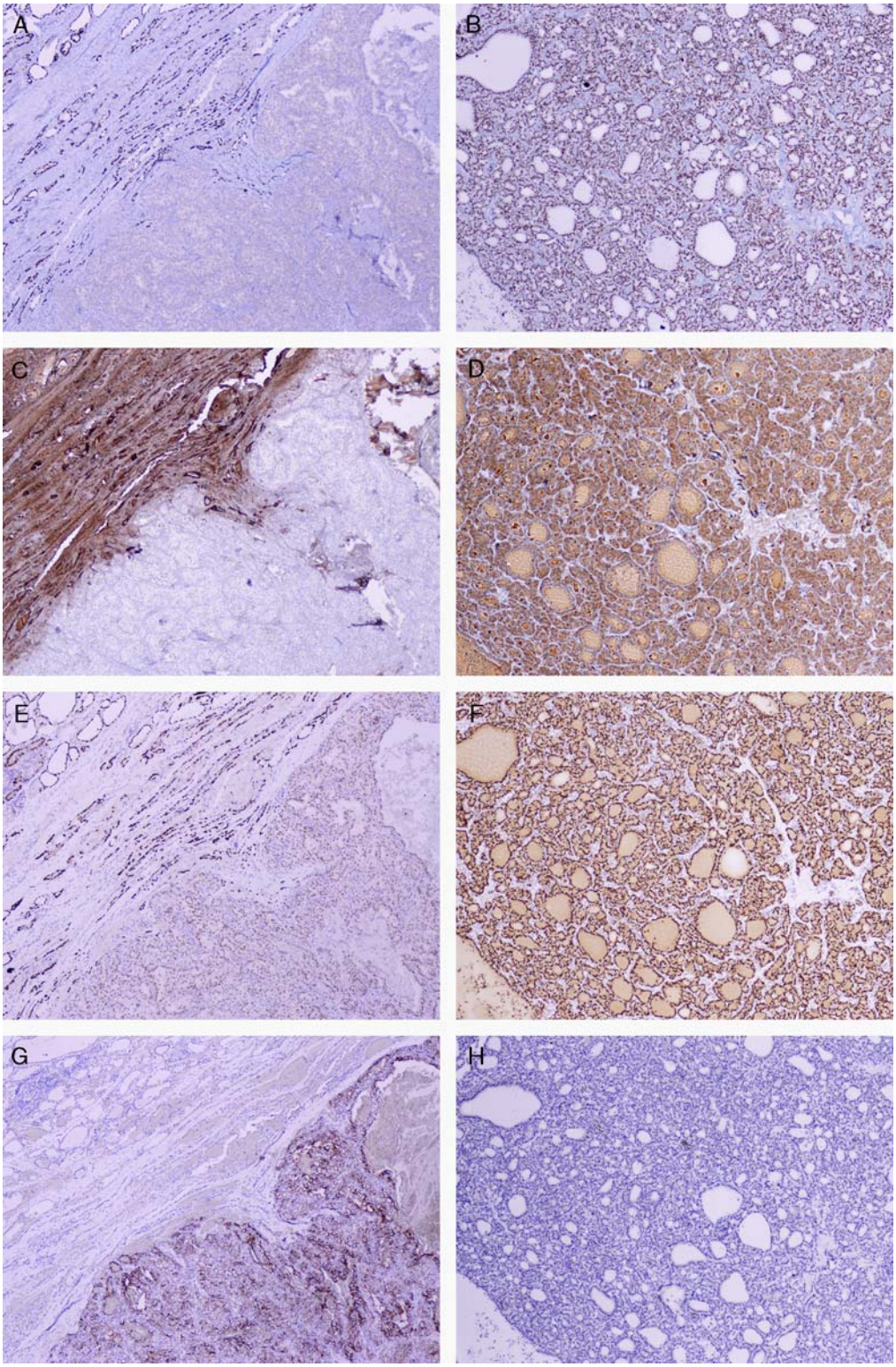

FIGURE 2. Immunostaining patterns of metastatic ccRCC (left) of the thyroid and a thyroid adenomatoid nodule (AN, right). TTF-1 is negative in ccRCC (A) but positive in AN (B); TGB is negative in ccRCC (C) but positive in AN (D); PAX8 is positive in ccRCC but more strongly positive in the surrounding normal thyroid tissue (E) and in AN (F); CAIX is positive in ccRCC (G) but negative in AN (H) (× 5). full color online

prominent clear cytoplasm and fine vasculature, as ccRCC may present as an isolated metastasis with an occult or remote primary.[16] The potential use of the immunopanel of TTF, TGB, and CAIX in thyroid FNAs will be explored in a separate study.

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