Primary Thyroid Carcinoma with Low-Risk Histology and Distant Metastases: Clinicopathologic and Molecular Characteristics

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Background: Distant metastases (DM) are a rare occurrence in well-differentiated thyroid carcinoma. The aim of this study was to analyze the clinical, pathologic, and molecular features of primary thyroid carcinoma with low-risk histology that develop DM.

Methods: A detailed clinicopathologic review and targeted next-generation sequencing were performed on a cohort of well-differentiated thyroid carcinoma lacking gross extrathyroidal extension, extensive vascular invasion, or significant lymph node metastases but exhibiting DM.

Results: Primary well-differentiated thyroid carcinoma with low-risk histologic features and DM was a rare occurrence, accounting for only 3% of metastatic non-anaplastic thyroid carcinoma. All 15 cases meeting the inclusion criteria harbored DM at presentation. The majority (11/15) of these tumors were follicular variant of papillary thyroid carcinoma (PTC), especially the encapsulated form ($n=8$). The remaining patients harbored encapsulated Hürthle cell carcinoma ($n=2$), encapsulated follicular carcinoma ($n=1$), and an encapsulated papillary carcinoma classical variant ($n=1$). Of the 12 encapsulated carcinomas, 10 had capsular invasion only and no vascular invasion. Ninety-two percent of the tumors exhibited extensive intra-tumoral fibrosis. Among the eight tumors that were subjected to next-generation sequencing analysis, a *RAS* mutation was the main driver (5/8), and *TERT* promoter mutation was highly prevalent (6/8). In four cases, *TERT* promoter mutations were associated with *RAS* or *BRAF* mutations. *BRAF*-mutated classical variant of papillary carcinoma also presented with DM but was less common (1/8). In 11/15 cases, the clinician was able to diagnose distant disease based on the clinical presentation. In 3/4 incidental cases that were genotyped, *TERT* promoter mutations were found.

Conclusions: When DM occur in primary thyroid carcinoma with low-risk histology, they are almost always found at presentation. The majority are encapsulated follicular variant of PTC with capsular invasion only. *TERT* promoter mutations occur at a higher rate than that seen in PTC in general and may help explain the aggressive behavior of these histologically deceptive primary carcinomas.

Keywords: distant metastasis, follicular variant, papillary thyroid carcinoma, Hürthle cell carcinoma, *RAS*, *TERT*

Introduction

OVER THE PAST THREE DECADES, the incidence of thyroid carcinoma in the United States has nearly tripled, with 62,980 new cases diagnosed in 2014 (1). In contrast, the five-year mortality rate has steadily declined from 8–10% in the 1970s to 2–3% in the 2000s, indicating the highly indolent clinical behavior of the vast majority of thyroid cancers (1). Distant metastases (DM) are an infrequent occurrence in non-anaplastic follicular cell-derived thyroid carcinoma (non-ANA FCDC), with a reported frequency of 1.2–13% (2–8). Unlike organ-confined low-stage non-ANA FCDCs, which

are usually associated with an excellent prognosis, older patients with DM carry a significant risk of death, with a five-year overall survival of 50–84% (4,5,9–11) and a five-year disease-specific survival of 58–80% (3,5,6,11). Indeed, in a study from the United States based on the Surveillance, Epidemiology, and End Results database including 9904 patients diagnosed with papillary thyroid carcinoma (PTC), the presence of DM is an independent predictor for poor overall survival (12). Additionally, DM is proven to be the most common immediate cause of death in non-ANA FCDC (13–15). The vast majority of non-ANA FCDC patients who die of thyroid cancer have aggressive histologic features in

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their primary tumors, such as a poorly differentiated component, gross extrathyroidal extension (ETE), and extensive vascular invasion (EVI) (15). However, there are rare cases where the patients harbor DM, despite the finding of a histologically low-risk primary tumor. These unusual bad actors have to the best of the authors' knowledge never been comprehensively studied at the histopathologic and molecular levels. By analyzing these rare tumors, this study hoped to find clinicopathologic and molecular markers that unmask the behavior of these "wolves in sheep clothing."

Materials and Methods

Study population

The study was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center (MSKCC, New York, NY). The institutional database was searched for patients diagnosed, treated, and followed by the endocrinology service at MSKCC for metastatic non-ANA FCDC between 1983 and 2009. These individuals developed DM either at the initial presentation or during clinical follow-up. A total of 123 patients with adequate pathologic data and clinical information were identified from this database and were included in the present study. Additionally, 11 cases of well-differentiated primary thyroid carcinomas with low-risk histology and DM were retrieved from the MSKCC pathology database and were also included.

Definition of primary thyroid carcinomas with low-risk histology

Primary well-differentiated FCDC without evidence of poorly differentiated component, EVI, gross ETE beyond the thyroid, and/or significant amount of nodal metastasis to neck lymph nodes were considered as low-risk histology. Thus, the histotypes included were PTC, follicular carcinoma (FTC), and Hürthle cell carcinoma (HCC). Significant metastatic lymph node disease was defined as pathologically confirmed metastatic thyroid carcinoma to more than five cervical lymph nodes and/or a metastatic lymph node measuring at least 1 cm in greatest dimension. These tumors were subjected to a meticulous histopathologic re-review by two endocrine pathologists (R.G. and B.X.).

Pathology review

Tumor size was measured as the maximal diameter of the resected tumor specimen. Mitotic rate was determined by counting 10 high power fields (HPFs, 400 $\times$, field size 0.24 mm²) with an Olympus microscope (U-DO model BX41; Olympus America, Inc., Center Valley, PA) in the areas of greatest concentration of mitotic figures. Capsular invasion was defined as complete penetration of the capsule by the tumor, and the number of these foci was recorded. The presence of vascular invasion was noted only when such foci were present within or beyond the capsule in accordance with criteria outlined by the Armed Forces Institute of Pathology (AFIP) fascicle (16). Briefly, only when the invasive focus protruded into the lumen of the vessel in a polypoid manner covered by endothelial cells, or when it was attached to the vessel wall or associated with thrombus formation, was it considered true vascular invasion. Areas of vascular invasion that were closely adjacent to one another were counted as

separate foci. The capsular and vascular invasion was subdivided into two categories: focal (<4 invasive foci) and extensive (≥ 4 foci). The presence or absence of microscopic ETE into the perithyroid soft tissue stroma was documented. Microscopic resection margins were categorized as positive (tumor at the inked margin) or negative (no tumor at the inked margin). Finally, when a regional lymph node sampling was performed during the initial surgery, the number of lymph nodes, metastatic status, size, presence of extranodal extension, and histological type of the metastasis were also recorded. Poorly differentiated carcinoma (PDC) was defined using the MSKCC criteria (17). In brief, a diagnosis of PDC was rendered when a tumor exhibited tumoral necrosis or elevated mitotic index of ≥ 5 mitotic figures per 10 HPFs (400 $\times$), regardless of the growth pattern and nuclear features.

Clinical review

The patient's charts were reviewed to record the following clinical parameters: age at diagnosis, sex, serum thyroglobulin (Tg) level at presentation and after total thyroidectomy, serum thyrotropin (TSH) levels after thyroidectomy, timing of the DM (i.e., at the initial presentation or during clinical follow-ups), site(s) of DM, presenting symptoms especially those attributed to DM, and disease status at the last follow-up. Additionally, gross extension beyond the thyroid (GET), defined as any appreciable gross adhesion and/or frank invasion of the primary thyroid carcinoma or metastatic carcinoma from a neck lymph node to the adjacent structures observed during the initial operation and confirmed histologically, was documented.

Molecular analysis

DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tumor sections and matched benign tissue. Eight primary well-differentiated thyroid carcinoma with low-risk histology and adequate DNA material were subjected to massive parallel high-output sequencing using MSKCC-IMPACTTM (MSKCC Integrated Mutation Profiling of Actionable Cancer Targets; MSKCC, New York, NY) platform, which is a hybridization capture-based assay for targeted deep sequencing of all exons and selected introns of 410 key cancer genes, encompassing the majority of established oncogenes and tumor suppressor genes (18).

Results

From 123 patients with non-ANA FCDC with DM, 74 (60%) patients had DM at the time of initial clinical presentation, while the remaining 49 (40%) developed DM during follow-ups. The vast majority (97%) of patients had at least one of the following high-risk features: a pathologic diagnosis of PDC ($n=65$; 53%), EVI ($n=62$; 51%), GET documented during the initial thyroidectomy $\pm$ neck dissection ($n=21$; 17%), and/or significant volume of metastatic disease in the neck lymph nodes (i.e., >5 lymph nodes and/or the largest involved lymph node >1 cm in size; $n=4$; 3%; Fig. 1). In contrast, only 4/123 (3%) patients had a well-differentiated carcinoma without the above-mentioned high-risk histologic features in their primary. An additional 11 patients were also retrieved from the MSKCC pathology database (outside the data limit of the endocrinology

FIG. 1. Workflow, methodology, and characteristics of the study cohort, which encompassed 123 patients with differentiated follicular cell–derived carcinoma and distant metastasis (either at clinical presentation or developed during follow-up). PDC, poorly differentiated carcinoma; EVI, extensive vascular invasion; GET, gross extension beyond the thyroid; LN, lymph node; DM, distant metastasis.

database, as well as cases referred to the hospital) who developed DM and were diagnosed with well-differentiated thyroid carcinomas without PDC, EVI, GET, and significant nodal disease. In total, 15 patients had low-risk histology in their primaries and DM. In all 15 cases, DM was found at initial presentation.

Clinicopathologic characteristics

The clinicopathologic features of these 15 well-differentiated primary thyroid carcinomas without high-risk features and with DM at presentation are summarized in Tables 1 and 2. These tumors occurred in older patients (median age of presentation = 63 years; range 50–75 years). There was a female predominance, with a female to male ratio of 2.75:1. In regard to sampling, a median of 2.2 blocks/cm of tumor was examined (range 1–5 blocks/cm of tumor). A median of 14 slides per thyroidectomy specimen was examined (range 3–43 slides). The majority ($n=8$) of these tumors were encapsulated follicular variant of PTC with invasion (EFVPTC), while the remaining patients were diagnosed as infiltrative follicular variant of PTC (IFVPTC; $n=2$), papillary microcarcinoma infiltrative follicular variant ($n=1$), encapsulated PTC classical variant ($n=1$), encapsulated FTC ($n=1$), and encapsulated HCC ($n=2$). Most of the patients had small tumors ≤ 2 cm in size (AJCC pT1, $n=10$) without lymphovascular invasion ($n=12$). All 15 cases had negative surgical margins and exhibited no evidence of microscopic ETE. Twelve tumors were encapsulated, while the remaining three were partially encapsulated or lacked a capsule completely. All 12 completely encapsulated carcinomas exhibited capsular invasion, either focal ($n=9$) or extensive ($n=3$), with foci ranging from one to seven per case. Ten of the 12 encapsulated carcinomas displayed capsular invasion only without vascular invasion. The number of foci of vascular invasion was as follows: absent in 10 cases, one focus in one patient, and two foci in another. One case

displayed fewer than four foci at the initial routine diagnostic evaluation, but the exact number of foci could not be counted, since the slides were sent back to the original institution at the time of the study (Table 2). There were three tumors with only one focus of capsular invasion, no vascular invasion, and DM. The only patient who had nodal metastasis at the initial operation harbored a completely encapsulated classical variant of PTC that contained approximately 28% of tall cells and 7% of columnar cells. Intra-tumoral fibrosis and calcification were not assessable in two patients whose slides were sent back to their original institution at the time of the study. Among the 13 tumors with assessable intra-tumoral fibrosis and calcification, 12 had extensive intra-tumoral fibrosis, and 10 showed calcifications (Fig. 2). Six of the 15 cases had multicentric thyroid carcinomas harboring one or more foci of tumor in the form of papillary microcarcinomas in addition to the index lesion. All cases had histologically confirmed DM. Four of the 15 cases had mild mitotic activity (<5 mitosis per 10 HPFs; 400). No mitosis was found in the remainder.

The median follow-up for these 15 cases was 32 months (range 2–158 months). Four cases had a documented fatal outcome: two from unknown causes, and two from metastatic thyroid carcinoma. The remaining 11 cases were alive with disease at the time of last follow-up. The DM were predominantly found in the bone ($n=12$), while metastasis to other sites were relatively rare, including the lung ($n=3$), chest wall ($n=2$), liver ($n=2$), brain ($n=1$), kidney ($n=1$), and pelvic and buttock soft tissue ($n=2$; Table 2). The majority of patients ($n=11$) sought clinical attention for symptoms related to DM (e.g., pain, pathological fractures, palpable mass, or cognitive dysfunction). Four patients were asymptomatic and presented with lesions in the bone or lung noted on radiological studies performed for unrelated reasons. Four of the 11 symptomatic patients and none of the four asymptomatic patients were deceased at the last follow-up. Nine of the 15 patients did not have interpretable

TABLE 1. CLINICOPATHOLOGIC CHARACTERISTICS OF THE 15 PATIENTS WITH LOW-RISK HISTOLOGY IN THEIR PRIMARIES AND DISTANT METASTASIS

		N
Total		15
Sex	Female	11
	Male	4
Age, median (range)		63 (50–75)
Size, median (range)		1.8 (0.2–5.5)
Diagnosis	FVPTC, infiltrative	2
	FVPTC, encapsulated	8
	Papillary microcarcinoma, infiltrative follicular variant	1
	PTC, classical variant	1
	HCC, encapsulated	2
	FTC, encapsulated	1
AJCC pT stage	T1a	1
	T1b	9
	T2	4
	T3	1
Encapsulation	Completely encapsulated	12
	Partially encapsulated	2
	No capsule	1
Capsular invasion for encapsulated carcinoma (n = 12)	Extensive	3
	Focal	9
Vascular invasion	Focal	3
	Absent	12
Intra-tumoral fibrosis (n = 13)	Present	12
	Absent	1
Margin status	Negative	15
	Positive	0
Microscopic extrathyroidal extension	Absent	15
	Present	0
Lymph node status	Not sampled	8
	Negative	6
	N1a	1
Distant metastasis at presentation	Present	15
	Absent	0
Follow up period (months), median (range)		32 (2–158)
Disease status at the last follow-up	Dead of unknown cause	2
	Dead of disease	2
	Alive with disease	11

AJCC, American Joint Committee on Cancer; PTC, papillary thyroid carcinoma; HCC, Hürthle cell carcinoma; FTC, follicular carcinoma.

postoperative serum Tg levels because of high or unavailable serum TSH (n = 4), high antithyroglobulin antibodies (n = 1), or simply because the serum Tg assay was not performed (n = 4). The six cases with assessable post-thyroidectomy serum Tg had low postoperative (i.e., <5 ng/mL) Tg values (1.4 and 4.9 ng/mL), as well as high ones (49, 2058, 6303, and 7570 ng/mL). Preoperative serum Tg values were available in five cases and were elevated (78, 79, 640, 9300, and 14,675 ng/mL). Out of the four cases discovered incidentally, the postoperative serum Tg was low in one patient (1.4 ng/mL), high in another (6303 ng/mL), while in the remaining two, it was not available or uninterpretable.

Molecular characteristics

Targeted next-generation sequencing was performed on eight tumors, including five EFVPTC, one IFVPTC, one PTC classical variant, and one HCC, and the results are summarized in Table 3. The median mutation burden was 3 (range

1–7), with 6/8 tumors having two or more mutations. *RAS* mutations were the main driver in this cohort, affecting 5/8 tumors tested, including four EFVPTC and one IFVPTC. Only one tumor, a classical variant of PTC, harbored a *BRAF*^{V600E} mutation. A *RET/PTC* rearrangement was not detected in this cohort. The two tumors that did not contain *RAS* or *BRAF* mutations were one EFVPTC and one HCC. Telomere reverse transcriptase (*TERT*) promoter mutations occurred at a high frequency (6/8) in this cohort. *TERT* promoter mutations co-occurred with either *BRAF* or *RAS* mutations in 4/6 tumors. A *EIF1AX* mutation was detected in one *NRAS*-mutated EFVPTC. Other mutations included *NTRK1*, *PIK3CA*, *ARID2*, *ATM*, *CDKN1A*, *FAT1*, *RBM10*, *BAP1*, *INHA*, *PTPRS*, *PTPRT*, and *BCL6*.

Discussion

DM are an infrequent occurrence in non-ANA FCDC, with a reported frequency of 1.2–13% (2–8). As patients with DM

TABLE 2. PRESENTING SYMPTOMS, HISTOLOGIC CHARACTERISTICS, SITES OF DISTANT METASTASES, AND MOLECULAR FINDINGS IN PATIENTS HARBORING PRIMARY TUMORS WITH LOW-RISK HISTOLOGY AND DISTANT METASTASIS

Pt	Age/ sex	Pathologic diagnosis	CI (no foci)	VI (no foci)	Tumor size (cm) ^b	Additional foci of papillary microcarcinoma	Sites of DM at presentation	Presenting symptoms	TERT promoter mutations	BRAF ^{V600E} / RAS mutations
1	71F	EFV/PTC	Focal (<4)	None	1.5	1	Bone	Incidental imaging findings	Present	None
2	58F	EFV/PTC	Extensive (4)	None	1.2	0	Chest wall, bone	Incidental imaging findings	Present	NRAS
3	66F	EFV/PTC	Extensive (6)	None	2.0	2	Lung, Bone	Back pain	Present	NRAS
4	75M	EFV/PTC	Focal (2)	None	4.0	0	Bone, buttocks soft tissue	Pain and sore spot on buttock	Absent	HRAS
5	57F	EFV/PTC	Focal (2)	None	2.0	3	Bone	Back pain	Absent	NRAS
6	66F	EFV/PTC	Focal (1)	None	1.5	0	Bone, lung	Shoulder pain	NA	NA
7	57F	EFV/PTC	Focal (3)	None	2.25	0	Bone	Pathologic fracture	NA	NA
8	52F	EFV/PTC	Focal (<4)	Focal (2)	2.7	2	Liver, bone	Abdominal pain	NA	NA
9	66M	IFV/PTC	NA ^a	None	2.5	0	Bone	Clavicle mass	Present	NRAS
10	50F	IFV/PTC	NA ^a	Focal (<4)	1.8	0	Chest wall, bone	Chest wall mass	NA	NA
11	66F	PMC, IFV	NA ^a	None	0.2	3	Bone	Incidental imaging findings	NA	NA
12	54M	PTC, classical variant, encapsulated	Extensive (7)	None	1.7	2	Lung	Incidental imaging findings	Present	BRAF ^{V600E}
13	59F	EFC	Focal (2)	Focal (1)	1.1	0	Bone	Hip pain	NA	NA
14	70M	HCC	Focal (1)	None	5.5	0	Brain	Cognitive dysfunction	Present	None
15	63F	HCC	Focal (1)	None	1.0	0	Bone	Hip pain	NA	NA

^aCapsular invasion was not assessable, since tumor lacked a complete capsule.

^bTumor size was the greatest diameter of the largest carcinoma focus.

AWD, alive with disease; CI, capsular invasion; DM, distant metastasis; DOD, dead of disease; DOUC, dead of unknown cause; EFC, encapsulated follicular carcinoma; EFV/PTC, encapsulated follicular variant of papillary thyroid carcinoma; F, female; FTC, follicular thyroid carcinoma; HCC, Hürthle cell carcinoma; IFV/PTC, infiltrative follicular variant of papillary thyroid carcinoma; M, male; NA, not applicable/not available; Pt, patient; PTC, papillary thyroid carcinoma; VI, vascular invasion.

FIG. 2. Microscopic pictures of encapsulated papillary thyroid carcinoma follicular variant with capsular invasion only and distant metastasis at presentation. (A) Low-power view (20 $\times$) showing marked intra-tumoral fibrosis (f). (B) Capsular invasion (CI) with complete penetration of tumor capsule (ca; 100 $\times$). (C) The tumor shows follicular growth (100 $\times$). (D) Nuclear features of papillary thyroid carcinoma: clear, enlarged, and irregular nuclei (arrows; 400 $\times$).

carry a significant risk of death, with a five-year overall survival of 50–84% (4,5,9–11) and a five-year disease-specific survival of 58–80% (3,5,6,11), it is of paramount importance to identify tumors at risk of developing DM at the time of initial presentation to assist clinical decision making.

Some published studies have suggested that the frequency of DM was different among the various histologic types, with HCC having the highest frequency, PTC the lowest, and FTC in between (7,8,19). Sugino *et al.* reported older age and larger tumor size as risk factors for DM in a cohort of 123 patients with FTC (20). In their study, the presence of vascular invasion was not associated with an increased risk of developing DM during follow-up. However, these authors did not evaluate the extent of vascular invasion. Recently, it has been shown that the vast majority of patients who die of non-ANA FCDC have aggressive histologic features in their primary tumors (15). A majority of these fatal carcinomas were associated with DM, which were the immediate cause of death in most fatal cases (13–15). Similarly, in the current series, the overwhelming majority of patients with DM had in

their primary tumors at least a poorly differentiated component, gross ETE, EVI, and/or a significant volume of lymph node metastases (e.g., more than five positive lymph nodes and/or one positive node >1 cm in greatest dimension). Together, these results suggest that such histologic features are useful tools to identify tumors at risk of developing distant disease and cancer-specific death. Hence, it is crucial to evaluate and report these parameters at presentation and take them into consideration for risk assessment and treatment planning of non-anaplastic thyroid carcinoma.

As shown in this study, primary tumors with low-risk histology (i.e., lacking the above aggressive features) also can give rise to DM. However, this is a rare cause of distant disease, since it was present in only 3% of the database of patients with DM.

A large proportion (12/15) of these apparently indolent primary tumors were encapsulated carcinomas, including eight encapsulated follicular variant of papillary carcinoma, two encapsulated HCCs, one encapsulated classical PTC, and one encapsulated FTC. Except for the encapsulated classical

TABLE 3. MUTATION PROFILE IN EIGHT PATIENTS WITH LOW-RISK HISTOLOGY IN THEIR PRIMARIES AND DISTANT METASTASES

Pt	RAS	BRAF	TERT promoter	Other mutations	Tumor type	Outcome
1			Mutated	<i>PIK3CA</i> , <i>ARID2</i> , <i>ATM</i> , <i>FAT1</i>	EFVPTC	AWD
2	<i>NRAS</i>		Mutated	<i>RBM10</i>	EFVPTC	AWD
3	<i>NRAS</i>		Mutated	<i>EIF1AX</i>	EFVPTC	AWD
4	<i>HRAS</i>		Wild type	<i>BAP1</i>	EFVPTC	DOUC
5	<i>NRAS</i>		Wild type		EFVPTC	AWD
9	<i>NRAS</i>		Mutated	<i>PTPRT</i> , <i>NTRK1</i> , <i>INHA</i> , <i>PTPRS</i> , <i>BCL6</i>	IFVPTC	AWD
12		<i>BRAF</i> ^{V600E}	Mutated		PTC, classical variant	AWD
14			Mutated	<i>CDKN1A</i> , <i>ARID2</i>	HCC	DOD

PTC case, none of the remaining 11 patients developed lymph node metastases. This is expected for the HCC and FTC, as these tumors have the tendency to develop distant rather than regional metastases (21). In regard to encapsulated FVPTC, previous studies have shown a molecular profile and clinical behavior similar to that of follicular adenoma or FTC (22,23). Genotypically, EFVPTCs harbor a high frequency of *RAS* mutations (36%) and a lack of *BRAF* mutations (0%) (23). Clinically, encapsulated FVPTCs with invasion, especially those with EVI, tend to give rise to distant rather than locoregional lymph node metastases (22). Consistent with these previous publications, the present study found that the *RAS* mutation occurred at high frequency (5/8), and was the main driver mutation in this cohort of low-risk well-differentiated thyroid carcinoma with DM. The *BRAF* mutation, on the other hand, was a rare event, affecting the only person with a classical variant PTC. The present study provides further evidence of the molecular similarity of EFVPTC with invasion to FTC and the potential capacity and propensity of DM in these tumors.

An intriguing finding was the presence of marked intra-tumoral fibrosis in a very high percentage of these cases. PTCs with intra-tumoral nodular fibrosis and heterotopic ossification were shown to have higher incidences of lymph node metastasis and extrathyroidal invasion than those without these features (24). In that study, higher expression of basic fibroblast growth factor (bFGF), bone morphogenetic protein-2 (BMP-2), and vascular endothelial growth factor (VEGF) was found in PTC with nodular fibrosis. Neovascularization was also increased in tumors with nodular fibrosis. The authors, however, did not study the impact of fibrosis on distant disease. Of note, marked intra-tumoral fibrosis and/or heterotopic ossification were detected in two of three previously reported papillary microcarcinomas that developed DM and caused death (25). It is tempting to speculate that fibrosis in these tumors is indeed a regressive change, and similar to melanoma, regression may lead to under-staging/under-grading of the tumor. Further work is needed to understand the mechanisms leading to marked tumor fibrosis in patients with DM and indolent histology.

A more perplex finding is the absence of vascular invasion in most cases (12/15), including 8/10 encapsulated tumors. Since encapsulated low-grade thyroid carcinomas with capsular invasion are known almost never to behave aggressively (26), it is nearly impossible for the pathologists to predict distant spread based on histology alone in these unusual cases.

TERT promoter mutation is an uncommon molecular event in PTC, being reported in 9% of 496 PTCs in The Cancer Genomic Atlas study (27). On the other hand, *TERT* promoter mutation occurs at a high frequency in PDC and anaplastic thyroid carcinoma, with 40% and 73%, respectively (28,29). In PTC, *TERT* promoter mutations have been shown to be a molecular signature associated with aggressive clinical behaviors (e.g., propensity for regional and DM and disease-specific death) (30,31). Compared with the 9% mutation rate in PTC in general, *TERT* promoter mutation was highly prevalent (6/8) in this cohort of differentiated carcinoma with low-risk tumor morphology but DM. In four cases, *TERT* promoter mutations were associated with *RAS* or *BRAF* mutations. The co-existence of these two events has been shown to correlate with high risk of recurrence and to in-

crease disease-specific mortality in differentiated thyroid carcinomas (29). The above *TERT*-related findings may explain the aggressive behavior of these tumors. Importantly, it suggests that *TERT* can be used as a molecular marker indicative of aggressive clinical behavior in these histologically indolent primary tumors.

Mutation in the eukaryotic translation initiation factor 1A X-linked (*EIF1AX*) has been reported to co-occur with *RAS* mutation in well-differentiated, PDC, and anaplastic thyroid carcinoma, and it is an adverse molecular signature in PDC (28,32,33). Consistent with such findings, co-existence of *NRAS* and *EIF1AX* was detected in one EFVPTC with DM at presentation in the present cohort. One of the cases in the study showed mutation of *CDKN1A*, which is a tumor suppressor gene encoding p21. Interestingly, higher expression of p21 was found to be associated with high-risk clinical features in PTC (34). Other notable alterations were those of *ARID2*, a member of the SWI/SNF remodeling complex, and *ATM*, a tumor suppressor gene. Both of these genes have been found to be mutated in PDC and anaplastic carcinomas (31). Most of the remaining somatic mutations found such as *RBM10*, *FAT1*, *BAP1*, *BCL6*, *PTPR*, *PTPRS*, and *INHA* were seen in only one case and have not been reported in thyroid carcinomas.

From a practical point of view, the important question centers on how to identify aggressive behavior in these histologically indolent primary tumors. In the vast majority of cases (11/15), the clinician was able to diagnose distant disease based on the patient clinical presentation (e.g., bone fracture). There were, however, four cases where DM were detected incidentally by imaging ordered for non-thyroid diseases. In one of those patients, the endocrinologist would have suspected metastatic disease based on a high postoperative serum Tg (6303 ng/mL) had the patient being subjected to surgery before the discovery of distant disease. There was, however, another incidental case where the postoperative serum Tg was low (1.4 ng/mL). The aggressive behavior of this patient's tumor would in theory have been unrecognized by the clinician and pathologist based on the histology and Tg level. Three of these four incidental cases had tissue for genotyping. Interestingly, *TERT* promoter mutations were found in each of the genotyped cases and were associated with a *RAS* mutation in one individual, mutation in *PIK3CA* in another, and *BRAF*^{V600E} in the patient with low postoperative serum Tg. The detection of *TERT* mutations seems to have the potential of unmasking the aggressive potential of these low-grade tumors.

In conclusion, this study demonstrates the power of meticulous, clinical, operative, and routine histopathologic examination in stratifying differentiated thyroid carcinomas. One can recognize patients with non-ANA FCDC at risk of DM based on the presence of the following four clinicopathologic features in the primary resection: GET, EVI, a histologic diagnosis of PDC, and significant lymph node metastasis involving more than five lymph nodes and at least one >1 cm in greatest dimension. Non-ANA FCDCs without these four clinicopathologic features very rarely develop DM. If DM does occur in such lesions, it is commonly present at the time of the initial clinical evaluation. In the vast majority of cases, the clinical presentation will uncover the DM associated with these indolent-appearing primary tumors. These tumors are enriched in *RAS*-mutated FVPTC and

harbor a very high rate of *TERT* promoter mutations. The latter genetic alteration may help identify aggressive behavior in patients with these histologically low-risk primaries, including those extremely infrequent cases with asymptomatic DM.

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