Published in final edited form as:

Am J Surg Pathol. 2017 April ; 41(4): 446–457. doi:10.1097/PAS.0000000000000814.

Clinical and Morphologic Features of ETV6-NTRK3 Translocated Papillary Thyroid Carcinoma in an Adult Population without Radiation Exposure

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Abstract

The ETV6-NTRK3 translocation characterizes a subset of radiation associated and pediatric papillary thyroid carcinomas (PTC). We now describe the clinicopathologic features of ETV6-NTRK3 translocated PTC in an adult population without radiation exposure. Twelve cases were identified by next generation sequencing (ThyroSeq v2). Mean patient age was 37 years with a female predilection (10:2). Preoperative fine needle aspiration was performed on 6 patients of which 4 were classified as “malignant,” while two were classified as “follicular lesion of undetermined significance.” One third (4/12) of patients demonstrated extrathyroidal extension and one half of patients (5/10) demonstrated lymph node metastases. One patient presented with brain metastasis. Tumors typically (8/12) demonstrated an admixture of follicular and papillary patterns and were usually infiltrative and multinodular (6/12 cases). Tumors often showed clear cell or oncocytic foci and demonstrated overt nuclear features of PTC, though characteristically, interspersed bland areas were common, even in metastases. Cytoplasmic vacuolization resembling that of mammary analogue secretory carcinoma was also common but focal. TTF1 was positive and S100 was negative in all tested cases confirming a thyroid phenotype. Unique morphologies included glomeruloid follicles, reverse polarization of nuclei. Survey of the TCGA datasets revealed similar findings. Thus, *ETV6-NTRK3* translocated PTC are locoregionally aggressive and can metastasize distantly. They are characterized by mixture of papillary and follicular architecture and may show cytoplasmic vacuolization akin to other *ETV6* translocated carcinomas. While nuclear features are typically overt, interspersed bland regions may cause diagnostic difficulty in metastatic sites, and may explain discordance on fine needle aspiration.

Keywords

Papillary thyroid carcinoma; *ETV6-NTRK3*; next generation sequencing; translocation; TCGA

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INTRODUCTION

The incidence of thyroid carcinoma continues to increase due to multiple factors, including enhanced detection[1–3]. Papillary thyroid carcinoma (PTC) is the overwhelmingly dominant contributor to this phenomenon, representing about 80% of all thyroid carcinomas. The majority of PTC have a favorable outcome with a 5 year survival of over 95%, though age, tumor size, multifocality, nodal and distant metastases can identify some more aggressive cases[4]. In addition to staging parameters, PTC behavior is in part determined by morphologic subtype or variant. Some are more aggressive, notably tall cell variant[5], columnar cell variant[6], and the newly described, hobnail variant,[7] while others, notably encapsulated follicular variant of PTC, are far more indolent[8,9]. The distribution of these morphologic variants has changed over the past 3 decades,[10] and the understanding of molecular alterations that correspond to many of these variant morphologies has improved.

Recently, the cancer genome atlas (TCGA) had surveyed the molecular landscape of 496 PTC. This survey confirmed the importance of established somatic mutations in *BRAF* and *RAS* genes, and known fusions involving *RET* and *NTRK1.* It additionally confirmed morphologic correlates, namely that conventional and tall cell variants of PTC tended cluster within the $BRAF^{V600E}$ positive (or associated) set of tumors while follicular variants of PTC clustered within the *RAS positive* set of tumors[11]. The TCGA study established several novel driver mutations and fusions as well, including previously described *NTRK3* associated translocations, [12,13] albeit with low prevalence [14]. While provisional attempts were made to determine by genetic and epigenetic clusters whether these tumors were more "$BRAF^{V600E}$-like" or "RAS-like," the actual clinicopathologic categorization of these new molecular alterations has not been established.

Both *NTRK1* and *NTRK3* are of interest in thyroid carcinomas given the emerging potential to treat patients with TRK inhibitors such as LOXO-101 or entrectinib.[15,16] *NTRK3* is usually fused with *ETV6* in thyroid cancers. *ETV6-NTRK3* translocations have been described in a variety of tumors including secretory carcinoma of breast, mammary analogue secretory carcinoma of salivary gland, infantile fibrosarcoma, chronic eosinophilic leukemias, and acute myelogenous leukemias,[12,17] and in thyroid it actually represents the second most common rearrangement seen in the post-radiation setting, after *RET-PTC.* [12] In the adult population without prior history of irradiation, the clinicopathologic features of *ETV6-NTRK3* translocated PTC are not well characterized. We report our experience with a series of papillary thyroid carcinomas harboring this translocation.

MATERIALS AND METHODS

This study has been approved by the University of Pittsburgh Institutional Review Board (IRB: #991206).

Patient Selection

Case slides and/or FFPE tissue blocks were available in 12 of 44 cases with known *ETV6-NTRK3* translocation tested at the University of Pittsburgh Medical Center (2004–2015). None of these cases demonstrated additional molecular alterations. Seven cases were

identified retrospectively as part of a prior validation[18–20], while the remaining 5 cases were identified prospectively (2013–2015). Three of the six retrospectively examined cases were included in a prior series from our institution, though their case specific clinicopathologic features were not previously described [12]. For one case (case 7) both the primary and metastasis were tested and both harbored the translocation. All cases were in the adult population.

Pathologic Examination

Slides were reviewed by three authors (Y.E.N, S.I.C, and R.R.S); median number of slides review per tumor was 4.5 (range: 1 – 8). Clinicopathologic parameters were recorded. In addition to standard reporting parameters[21], the following features were documented: tumor border (encapsulated, capsular invasion, multinodular, diffusely infiltrative); calcifications (dystrophic or psammoma body); growth pattern (papillary, follicular, solid, trabecular); cytoplasmic characteristics (oncocytic, clear cell, vacuolated); nuclear features and distribution (size, overlap, membrane irregularities including pseudoinclusions and chromatin clearing); mitotic counts, and necrosis.

Molecular Analysis

Cases were evaluated by next-generation sequencing (DNA/RNA ThyroSeq v2 on Ion Torrent Personal Genome Machine, Carlsbad, Calif) for mutational testing of > 300 hotspots in 14 genes as well as 42 gene fusions as previously described[18–20]. Briefly, two libraries were prepared to study separately (i) point mutations and small indels using isolated DNA, and (ii) gene fusions and gene expression controls using isolated RNA.

The presence of at least 50 reads of the fusion transcript constituting 0.1% or more of all mapped sequencing reads for a given tumor was required to consider the test positive.

Gene expression analysis of 8 genes was used as part of the RNA panel to estimate the quantity of all types of cells (PGK1 gene), thyroid follicular cells (TG, TTF1, SLCA5, KRT7), C-cells and medullary thyroid carcinoma (CALCA), and parathyroid tissues (PTH) and to assess for additional coverage of nonthyroidal epithelial cells (KRT20) present in the specimen. The expression of thyroid follicular cell markers at levels >10% of all sequencing reads was used to determine that samples were adequate for molecular analysis.

Immunohistochemistry

Immunohistochemical stains for TTF-1 (clone:8G7G3/1; 1:50 dilution; Dako, Carpinteria, CA) and S100 (polyclonal rabbit; 1:500; Dako) were performed on 8 cases, and for HBME-1 (clone HBME-1; 1:100; Dako, Carpinteria, CA) and Galectin-3 (clone 9C4; prediluted; Ventana Medical Systems, Tucson, AZ, USA) on 6 cases using a Ventana Autostainer (Ventana Medical Systems, Tucson, AZ, USA). The antibody labeling for TTF-1 was performed using the avidin-biotin complex method and visualized using the i-VIEW 20 diaminobenzamide (DAB) detection kit (Ventana Medical Systems, Tucson, AZ, USA) with a brown chromogen substrate, while S100 was labeled using ultraVIEW alkaline phosphatase (AP) red detection kit (Ventana Medical Systems, Tucson, AZ, USA).

Data Extraction from the Cancer Genome Atlas (TCGA)

Data from the TCGA papillary thyroid carcinoma dataset were queried using cBioPortal http://www.cbioportal.org/ for cases with *NTRK3* translocations [22,23]. Clinical parameters were extracted and the scanned sections of tumor were reviewed in accordance to TCGA publication guidelines, https://cancergenome.nih.gov/publications/publicationguidelines.

RESULTS

Clinical, Cytologic and Staging Parameters

Clinical and staging parameters are summarized in Table 1. The mean patient age was 37 years (range: 19 – 60 years) with a female predominance (10:2). Most patients (7/8, 87.5%) were euthyroid, with one patient being hypothyroid on presentation. None of the patients with available information had a family history of thyroid cancer or personal history of prior irradiation. Fine needle aspirate biopsy (FNAB) findings were available on six patients. Two were designated as "follicular lesion of undetermined significance (Bethesda class III)," while four were called "malignant (Bethesda class VI)." All patients underwent total thyroidectomy and most (9/12, 75%) had at least some form of lymph node sampling, eight of whom actually had a formal central compartment dissection. Three patients also had a lateral neck dissection.

Mean tumor size was 3.3 cm (range: 1.4–5.9 cm). Four of 12 cases (33.3%) showed extrathyroidal extension and 8 of 12 (66.7%) had separate foci of papillary thyroid carcinoma. Nodal metastases were noted in half the patients with lymph node sampling (5/10) with 3 patients demonstrating lateral neck disease as well. Mean size of nodal metastases was 2.0 cm (range: 0.3 – 4.3 cm) with only one case demonstrating extranodal extension. One patient had a brain metastasis to the left parietal lobe on presentation. She was clinically suspected to have lung and bone metastases; radioactive iodine after dosimetry showed diffuse lung uptake, a probable proximal right humerus metastasis, and uptake in both sides of cervical adenopathy.

All patients received radioactive iodine. All patients were free of disease or with stable disease at time of last follow-up (median: 35 months, range: 3 – 94 months). The patient with brain metastasis did not have clinical evidence of structural recurrence at 16 months, but did demonstrate a persistently elevated thyroglobulin levels at 61.2 (1.3 – 31.8 ng/ml, normal range).

Histologic and Immunophenotypic Features

Histologic features are summarized in Table 2. None of the tumors were diffusely infiltrative, but one half of the tumors (6/12) demonstrated a multinodular permeative growth pattern with focal sclerosis (Figure 1a), while the remaining cases were uninodular and well demarcated/encapsulated. Most of the well demarcated encapsulated tumors however still demonstrated invasion; only two of these encapsulated tumors were negative for tumoral capsular and vascular invasion on available slides.

None of the encapsulated/well demarcated cases in which there was a nodal sampling demonstrated metastases, either nodal or distant. Lymphatic invasion was noted in 5/6 (83.3%) cases with multinodular growth (5/12, 41.7% overall). All these cases demonstrated nodal metastasis. Two cases (16.7%) demonstrated vascular invasion, including the case with brain metastasis.

Most (8/12, 66.7%) demonstrated a mixed follicular and papillary growth pattern, demonstrating at least 5% papillary growth with the multinodular permeative tumors tending to show more papillary components (Figure 1b). One case showed a predominantly (>90%) papillary growth pattern (Figure 2a). Solid growth pattern of at least 5% was noted in 3/12 (25%) cases. Psammoma bodies were noted in 3/12 (25%) cases (Figure 2b), and dystrophic calcifications were noted in 2/12 (16.7%) cases. Three cases showed less than 1% papillary growth. Of these, two were encapsulated and follicular patterned without tumoral capsular or angiolymphatic invasion (Figure 3a,b), while one case showed a mixture of follicular and solid growth pattern and focal tumoral capsular and vascular invasion. Two cases showed unusual glomeruloid/anastomosing follicular foci (Figure 4). Mitotic rates were minimal (range 0–1 per 10 hpf), and no case had coagulative tumor necrosis.

All tumors had foci with overt nuclear features (i.e. enlargement, overlap, membrane irregularities and chromatin changes) of papillary thyroid carcinoma, and all cases showed at least one nuclear pseudoinclusion on close scrutiny. However, tumors did typically demonstrate bland follicular patterned areas as well. Tumor metastases generally showed the same morphology as in the primary tumor. However, case 7 with brain metastasis (Figure 5a, 5b) and case 11 with nodal metastasis showed discordantly bland morphology.

While no defining cytonuclear features were noted, all tumors showed clear cell vacuolated foci (Figure 6a,b), and 5/12 (41.7%) cases showed >5% oncocytic features. Three of 12 cases showed a “reverse polarization” of nuclei (Figure 6c). Given the rare consideration of a metastatic secretory carcinoma or local extension from laryngotracheal minor salivary gland, which can mimic a primary thyroid neoplasm, we performed TTF-1 and S100. All 8 cases tested for TTF-1 were positive, and none of the cases showed S100 staining which confirms a thyroid follicular phenotype (Figure 7a–7c).

The background thyroid parenchyma in the majority of cases (8/12, 66.7%) showed chronic lymphocytic thyroiditis (Figure 7d) with a small proportion (3/12, 25%) showing nodular thyroid hyperplasia.

HBME-1 and Galectin-3 were both positive in all (6/6) cases tested. HBME-1 staining was more prominent in areas with overt nuclear features, and was attenuated or absent in areas of bland morphology (Figure 8a). In contrast, Galectin-3 was strongly positive in a diffuse, cytoplasmic and nuclear fashion (Figure 8b).

TCGA cases

Six cases with *NTRK3* rearrangements were identified. For five cases, the partner was *ETV6* while one case demonstrated an *RBPMS* partner. Clinical data were available for 5 cases, 4 with *ETV6-NTRK3* translocations, and one with *NTRK3-RBPMS* translocation.

For *ETV6-NTRK3* translocated cases, the mean patient age was 26 years (range: 17–36 years), and all patients were female. One case was pT1, and three cases were pT2. Only one case was N+. Median follow-up was 15 months (range: 0.1 to 79 months), and no recurrences were reported. Cases with *ETV6-NTRK3* gene rearrangement demonstrated similar morphologic features to the cases above in terms of multinodular growth pattern, and mixture of papillary and follicular growth patterns. Four of 5 cases also demonstrated scattered clear cell vacuolated foci, while one case was predominantly oncocytic. Reverse polarization was noted in two cases, and was prominent in the predominantly oncocytic case (Figure 9a). Three of 5 cases demonstrated a background of chronic lymphocytic thyroiditis.

One case with an *NTRK3-RBPMS* translocation was in a 20 year old female, and reported as a pT3N0 tumor. No recurrence was reported with disease free survival of 65 months. This tumor was uninodular and well demarcated and predominantly follicular patterned with only scattered papillae. This case did not have prominent clear cell or oncocytic changes and only focally showed anastomosing follicles (Figure 9b).

DISCUSSION

The *ETV6-NTRK3* translocation in thyroid carcinomas was noted by Ricarte-Fihlo et al [13] in 2 of 23 post-Chernobyl PTC patients. Similarly in a study by Leeman-Neil et al[12] of a cohort of 62 post-Chernobyl PTC, *ETV6-NTRK3* was identified in ~14% of cases, second in prevalence only to *RET/PTC* rearrangements. *In vitro* studies confirmed induction of these rearrangements by both I131 and γ-radiation. This prevalence was somewhat muted in the post-Fukushima young patient population, but still noted to be ~6%[24]. Interestingly, although data are limited, the prevalence of this translocation appears to be rather high in the sporadic (non-radiation associated) pediatric population as well, ranging from 7–22% [13,18,19]. When extended to a consecutive set of 151 of sporadic adult PTC in our local patient population, however, the prevalence was only 2%.[12] In the TCGA study, 1.2% of tumors demonstrated *NTRK3* associated fusions, either with *ETV6* or *RBPMS*.[11] Outside of this radiation association, *ETV6-NTRK3* translocated carcinomas are not well characterized.

Our limited clinical data yielded no definitive associations, specifically with regard to background thyroid disease or hereditary factors. But interestingly, histologically, most cases demonstrated some degree of chronic lymphocytic thyroiditis, though only one patient was actually documented to be hypothyroid. Prasad et al[18] did however identify a significant proportion of patients in their pediatric series with hypothyroidism and chronic lymphocytic thyroiditis. Picarsic et al[19] also noted ‘lymphocytic inflammation’ in three pediatric cases. Whether this background preceded the tumor or not is speculative.

This study reiterated some previously described pathologic findings, notably the predominant follicular growth pattern in *ETV6-NTRK3* translocated tumors. A good proportion showed solid growth as well[12,13,18]. However, it is critical to note that when stringent criteria are applied, most tumors do have a conventional papillary component. In fact, the prototypical appearance of these tumors is that of a mixed follicular and papillary architecture. One case was predominantly papillary, and only two cases could justifiably be designated as encapsulated follicular variants. Even here these two cases should be viewed

with caution as the lesional capsules were not entirely submitted. With the introduction of the new term non-invasive follicular thyroid neoplasm with papillary like nuclear features (NIFTP),[25] which is in essence a more indolent subset of encapsulated follicular variants of papillary thyroid carcinoma, this caveat is even more important, since these two cases would still not qualify as NIFTP due to incomplete lesional capsular examination. In fact, the high propensity for nodal disease, frequent finding of psammoma bodies, and also the relative ease at which diagnostic nuclear features were noted suggest a more 'conventional PTC' like profile for *ETV6-NTRK3* translocated cases.

Interesting findings in this study were areas of very deceptively bland morphology interspersed throughout primary tumors, and their predominance in metastatic foci in two cases. To some extent this heterogeneity is reflected by the preoperative FNAB where 2/6 cases were designated as follicular lesion of undetermined significance; these aspirates represented bland areas sampled within the tumors. While this phenomenon is not unique, it does highlight a potential pitfall as well as the importance of assessment of the primary tumor for accurate classification of thyroid cancers. These bland areas also showed attenuated HBME-1 staining, adding further to this pitfall. However, all tested cases showed strong and diffuse galectin-3 reactivity regardless of prominence of nuclear features. This is somewhat different from what is generally reported in the literature, where HBME-1 is considered to be more robust.[26,27] Mechanisms for this staining pattern are speculative as galectin-3 has a variety of roles including: cell-cell/cell-matrix adhesion, cell growth, neoplastic transformation/spread, cell cycle regulation/apoptosis, and cell repair processes.[27] A relationship between galectin-3 expression and the *ETV6-NTRK3* fusion product is not established.

Some other frequent features, notably the presence of clear cell/vacuolated areas and oncocytic change draw comparison to salivary type mammary analogue secretory carcinoma (MASC), and thus by association, secretory carcinoma of breast. Surprisingly, several examples of MASC arising in the thyroid region have emerged.[28–31] While origin and etiology are up for debate, when encountered, this could be mistaken for a primary follicular derived thyroid neoplasm. MASC demonstrate a vacuolated to eosinophilic cytoplasm as well as a mixture of papillary and follicular growth.[17] In fact, we have seen a case in consultation masquerading as a primary thyroid neoplasm[31], as well as another case arising from the trachea presenting as a thyroid mass (data unpublished). The fact that these share a translocation could be a potential pitfall. However, the morphologic mimicry is usually only focally noted, and most tumors have the appearance of a predominantly follicular patterned or conventional PTC. The follicular patterned areas *ETV6-NTRK3* translocated PTC show true colloid, and all cases were TTF-1 positive and S100 negative arguing against a relationship with salivary MASC. Limited follow up precludes any confident statements on level of aggression for *ETV6-NTRK3* translocated PTC other than the fairly high frequency of nodal disease on presentation akin to what was seen in the pediatric population.[18] We also document the first case with distant metastatic disease. The capacity for regional and distant spread does suggest a potential role for emerging targeted therapies that are currently in Phase 1 trial. [15,16]

Survey of the TCGA dataset demonstrates a younger age and more marked (complete) female predilection. However, age here is skewed by inclusion of a non-adult (17 year old) case, and in general the differences can readily be explained by the small number of cases in the dataset. Despite the availability only one digital slide per case, the morphology of *ETV6-NTRK3* translocated cases in this data set are fairly similar to that of our cases, including two cases with the unusual finding of reverse polarization, further supporting a characteristic spectrum of features for *ETV6-NTRK3* translocated tumors. With only one case with an *NTRK3-RBPMS* translocated case in this dataset, and none in our files, any commentary on clinicopathologic features for this variant translocation would be admittedly limited, but this case does not appear to demonstrate the same clear cell/vacuolated to oncocytic appearance that characterizes the *ETV6-NTRK3* translocated tumors.

In summary, *ETV6-NTRK3* translocated PTC is rare in the sporadic adult population. Similar to the pediatric population, a background chronic lymphocytic thyroiditis appears common. The most characteristic appearance is that of a mixed follicular and papillary growth pattern with frequent oncocytic and clear cell/vacuolated foci, as well as striking distributional heterogeneity of nuclear features ranging from overtly diagnostic to bland, even at metastatic sites. This heterogeneity of nuclear features may explain in a nonmalignant categorization on preoperative fine needle aspiration in 1/3 of our biopsied cases. While the focal secretory morphology is reminiscent of salivary MASC, other morphologic features (follicular growth with colloid), TTF-1 expression and absence of S100 expression confirm a thyroid follicular derivation. These tumors may show heterogeneous HBME-1 staining but strong Galectin-3 staining. Tumors are frequently locoregionally aggressive and can show distant metastasis. With the emergence of targeted therapies, recognition of this constellation of clinical and histologic findings may be helpful in suspecting this molecular alteration and suggest testing.

Acknowledgments

This study was supported in part by NIH grant CA88041 (Y.E.N)

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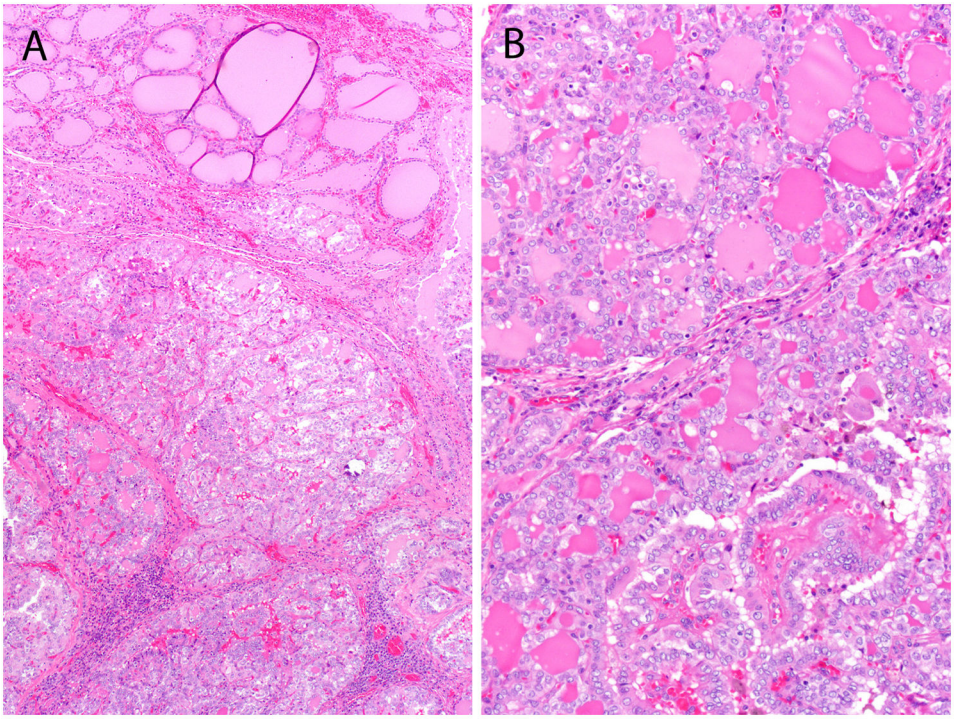

Figure 1.

Typical growth pattern and architecture in *ETV6-NTRK3* translocated PTC. a) Tumors are permeative in a multinodular fashion (H&E, 40×). b) Tumors typically demonstrate a mixture of papillary (bottom) and follicular (top) growth patterns (H&E, 200×).

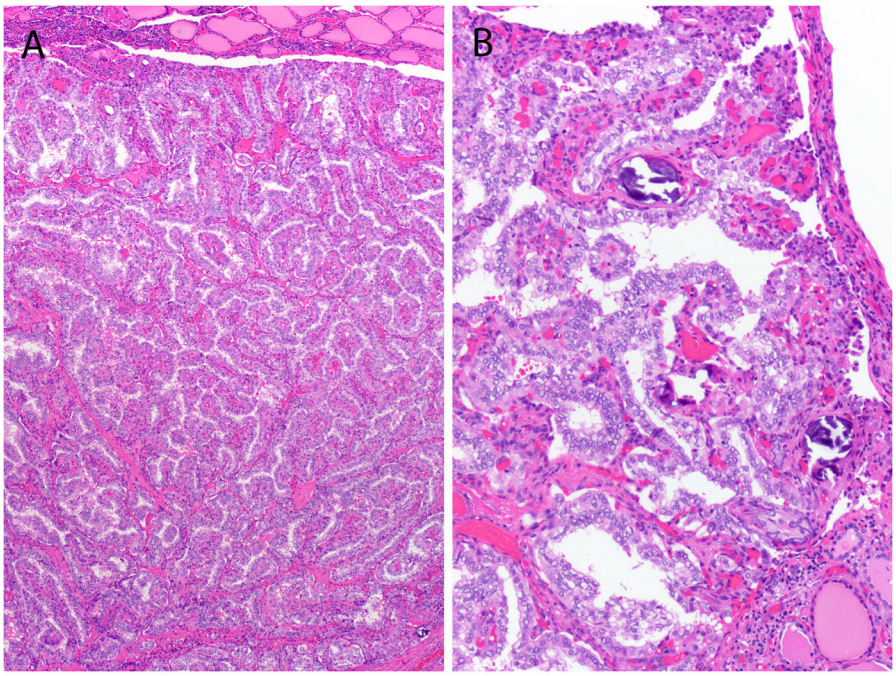

Figure 2.

ETV6-NTRK3 translocated PTC with a predominant papillary growth pattern. a) Papillae are arborizing and tightly packed (H&E, 40×). b) This tumor also demonstrates psammoma bodies (H&E, 200×).

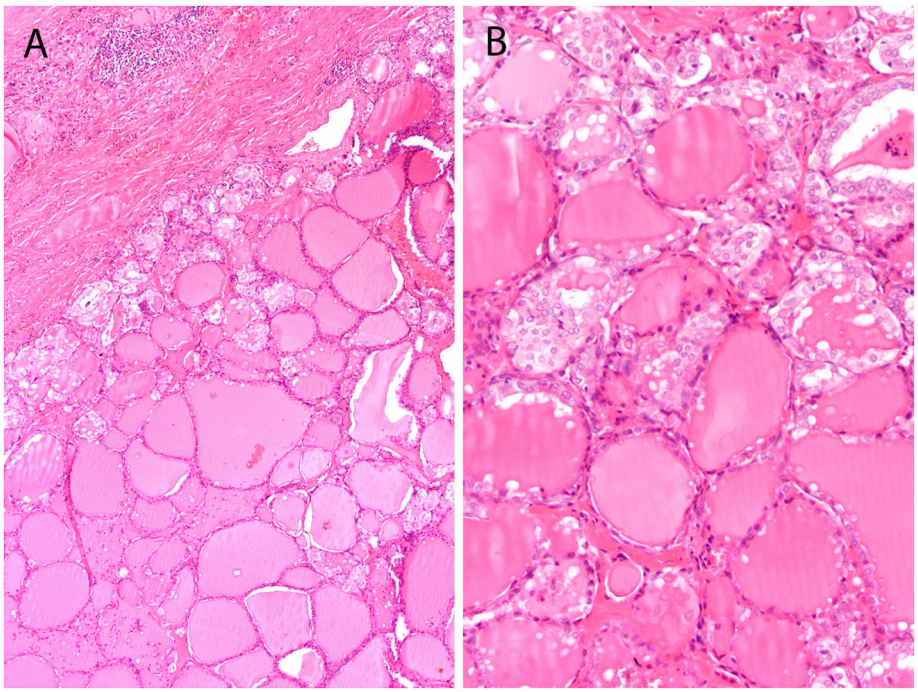

Figure 3.

Encapsulated predominantly follicular patterned tumors were rare. a) This encapsulated tumor shows only a sprinkling of foci, mainly underneath the capsule, with nuclear features of papillary thyroid carcinoma (H&E, 40×). b) Follicles with diagnostic nuclear features are interspersed between bland follicles with attenuated epithelium (H&E, 200×).

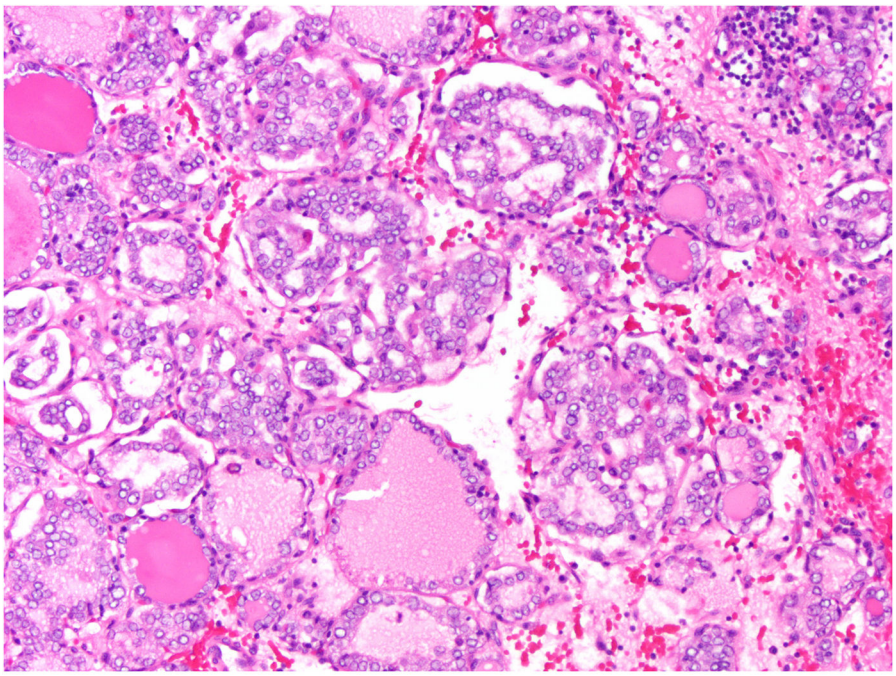

Figure 4.
In two cases follicles coalesced into glomeruloid/anastomosing foci (H&E, 100×).

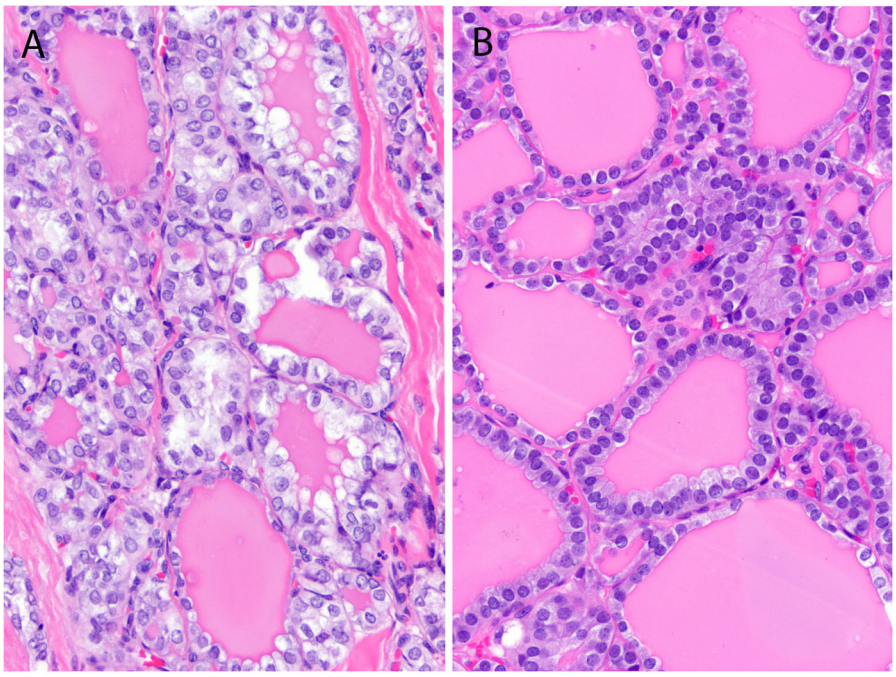

Figure 5.

Discordant morphology in between primary and metastasis in Case 7. a) Primary tumor shows fairly obvious nuclear enlargement, clearing and membrane irregularities (H&E, 200×). b) The metastasis to the brain, on the other hand, shows bland nuclei (H&E, 200×).

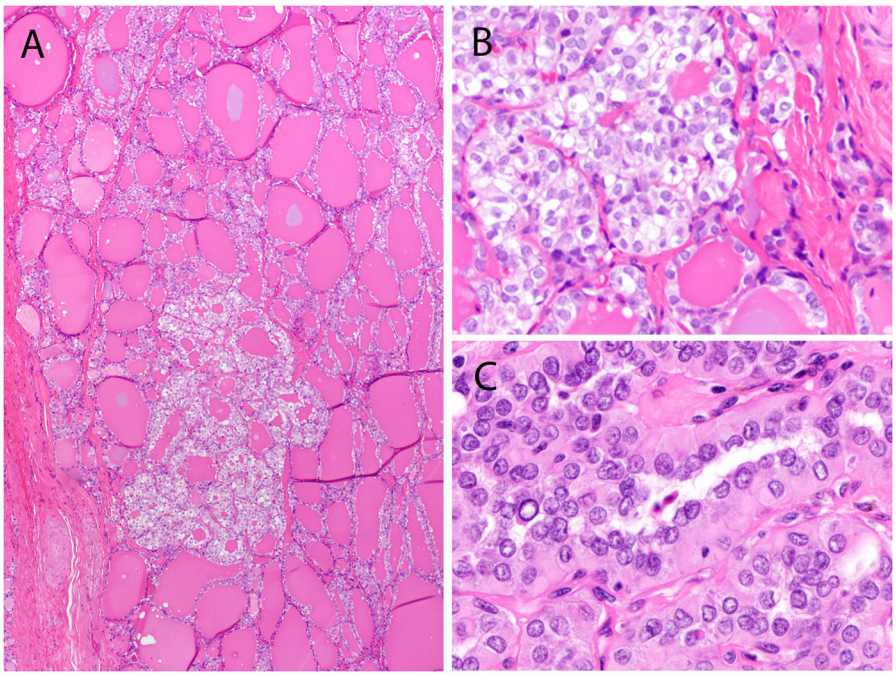

Figure 6.

Additional characteristic cytonuclear features in *ETV6-NTRK3* translocated PTC. a, b) Scattered clear cell foci are noted within all tumors (H&E, 20×, 200×). c) Oncocytic change was fairly common as well, and in some cases tumor nuclei lining follicles demonstrated a "reverse polarization." (H&E, 400×).

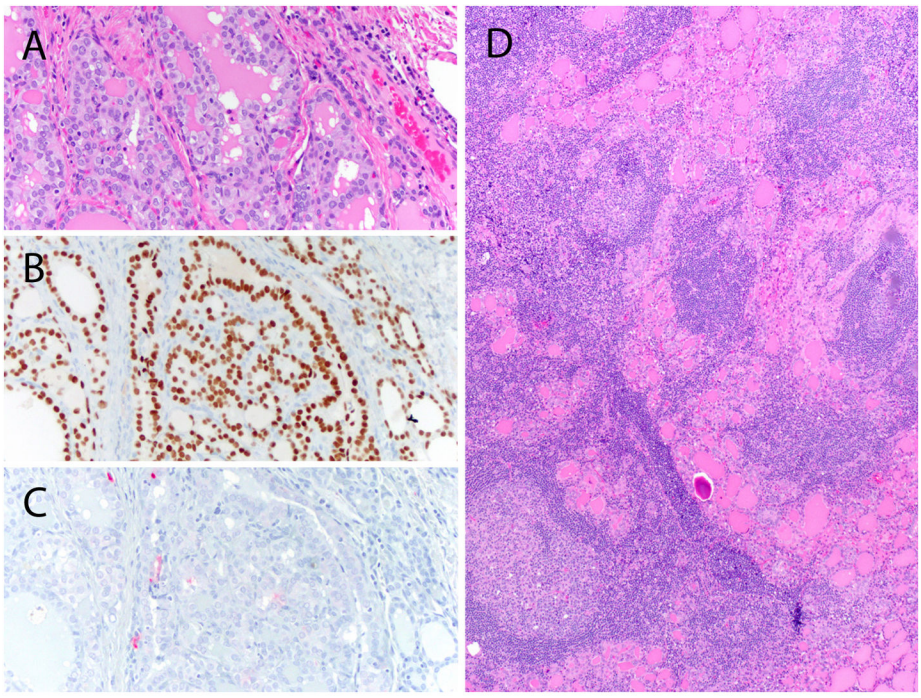

Figure 7.

Other features in *ETV6-NTRK3* translocated PTC. a) These tumors bear superficial resemblance to salivary mammary analog secretory carcinomas (H&E, 100×). b) However, TTF-1 is uniformly positive (100×), and c) S100 is uniformly negative (100×). d) Though most patients were euthyroid, chronic lymphocytic thyroiditis was common in the background thyroid parenchyma (H&E, 40×).

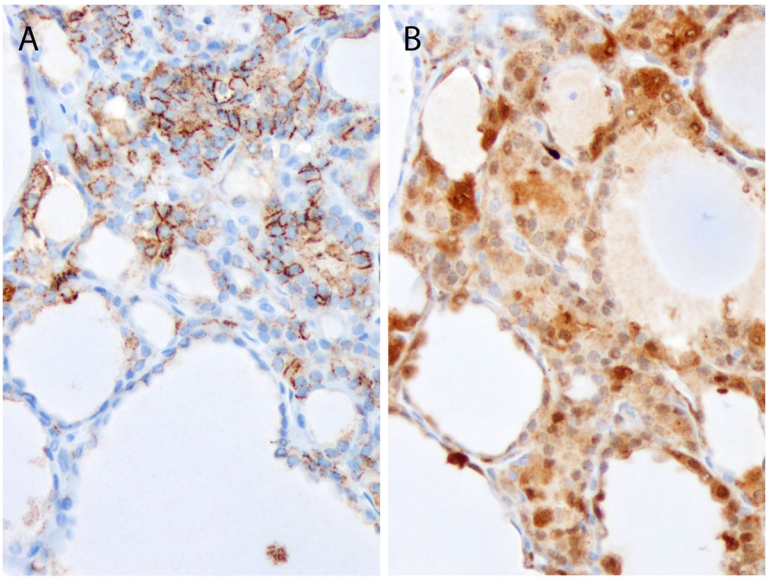

Figure 8.

HBME-1 and Galectin-3 staining profile in Case 7. a) HBME-1 staining is heterogeneous and attenuated to negative in areas without overt nuclear features of papillary thyroid carcinoma (200×). b) Galectin-3 however shows diffuse staining, both cytoplasmic and nuclear (200×).

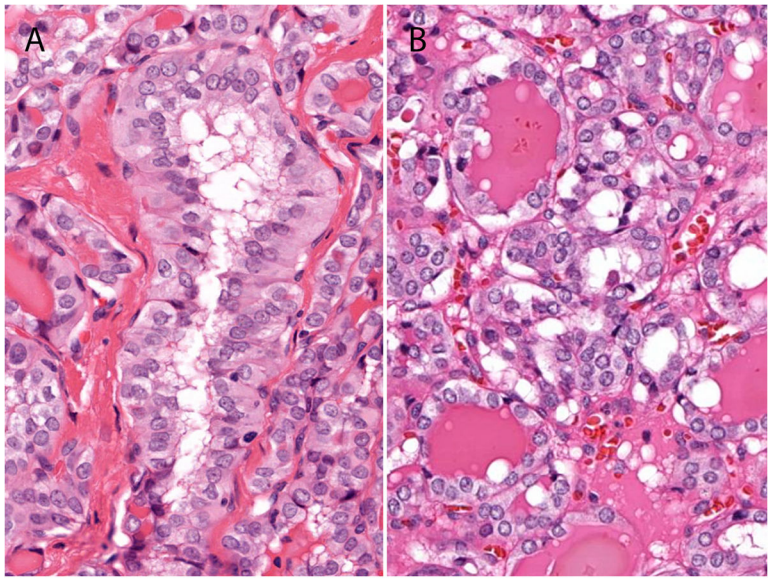

Figure 9.

TCGA case morphology. a) Despite limited review of a representative virtual slide reverse polarization of nuclei was noted in two cases with an *ETV6-NTRK3* translocation (case TCGA-E8-A438-01 shown here). b) *NTRK3-RBPMS* translocated case showing no significant unique morphology with perhaps the exception of vaguely anastomosing follicles (case TCGA-ET-A39L-01).

Table 1

Clinical and staging parameters for ETV6-NTRK3 translocated papillary thyroid carcinoma

Case	Age	Sex	FNAB	Type of Surgery	Functional Status	Family History of Thyroid Cancer	Prior History of Irradiation	Size (cm)	ETE	Multifocal/ Other PTC	pT	pN	pM	Largest Lymph Node Metastasis (cm)	Post-operative I131	Progression Free Survival (months)
1	21	F	Malignant, PTC	TT, CCND	Euthyroid	No	No	2.2	No	No	2	0			Yes	35
2	47	F		TT, CCND and LND	Euthyroid	No	No	5	No	Yes	3	1b		4.3	Yes	40
3	19	F	Malignant, PTC	TT, CCND and LND	Euthyroid	No	No	1.8	Yes	Yes	3	1b		3	Yes	38
4	22	F	Malignant, PTC	TT, CCND	Euthyroid	No	No	2.1	No	Yes	2	0			Yes	94
5	55	F		TT	Euthyroid	No	No	4.3	No	Yes	3	0			Yes	77
6	38	M		TT	n/a	n/a	n/a	5.9	No	Yes	3				Yes	
7	35	F		TT, CCND and LND	Euthyroid	No	No	5	Yes	No	3	1b	1*	2.4**	Yes	10
8	42	F		TT, CCND	Euthyroid	No	No	3	No	Yes	2	0			Yes	12
9	60	F		TT	n/a	n/a	n/a	2.6	Yes	No	3				Yes	88
10	38	F	LUS	TT	Euthyroid	No	No	1.4	No	Yes	1b				No	3
11	38	M	LUS	TT, CCND	Hypothyroid			3	Yes	Yes	3	1a		1.1	Yes	3
12	30	F	Malignant, PTC	TT, CCND	Euthyroid	No	No	3.5	No	No	2	1a		0.3	Yes	14

Abbreviations

* metastases to brain, lung and bone stable disease as of last follow-up

** extranodal extension was present

FNAB - fine needle aspiration biopsy PTC - papillary thyroid carcinoma LUS - lesion of undetermined significance TT - total thyroidectomy

CCND - central compartment neck dissection LND - lateral neck dissection n/a - not available ETE - extrathyroidal extension

Table 2

Histologic features of ETV6-NTRK3 translocated papillary thyroid carcinoma

Case	Border	LI	VI	Growth Pattern	Solid Component > 5%	Oncocytic Component >5%	Calcifications	Unusual Morphology	TTF-1	S100	Background Thyroid
1	Multinodular	No	No	Mixed Follicular/Papillary	No	No	No		Positive	Negative	mild CLT
2	Multinodular	Yes	No	Mixed Follicular/Papillary	No	Yes	Psammoma	Reverse Polarity of Nuclei			CLT
3	Multinodular	Yes	No	Mixed Follicular/Papillary	No	Yes	Psammoma		Positive	Negative	CLT
4	EWD with invasion	No	Yes	Follicular	Yes	Yes	No		Positive	Negative	CLT
5	EWD no invasion	No	No	Follicular	No	Yes	No	Glomeruloid/Anastomosing follicles	Positive	Negative	CLT, NTH
6	EWD no invasion	No	No	Follicular	No	No	No				CLT
7	Multinodular	Yes	Yes	Mixed Follicular/Papillary	No	No	No				
8	EWD with invasion	No	No	Mixed Follicular/Papillary	Yes	Yes	Dystrophic	Reverse Polarity of Nuclei			CLT, NTH
9	EWD with invasion	No	No	Mixed Follicular/Papillary	No	No	Dystrophic	Glomeruloid/Anastomosing follicles	Positive	Negative	NTH
10	EWD with invasion	No	No	Mixed Follicular/Papillary	No	No	No		Positive	Negative	CLT
11	Multinodular	Yes	No	Mixed Follicular/Papillary	Yes	No	No		Positive	Negative	CLT
12	Multinodular	Yes	No	Papillary	No	No	Psammoma	Reverse Polarity of Nuclei	Positive	Negative	

Abbreviations

EWD - Encapsulated/Well demarcated LI - Lymphatic invasion VI - Vascular invasion CLT - Chronic lymphocytic thyroiditis

NTH - Nodular thyroid hyperplasia