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A Detailed Clinicopathologic Study of ALK-translocated Papillary Thyroid Carcinoma

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Abstract: Pathogenic *ALK* translocations have been reported in papillary thyroid carcinoma (PTC). We developed and validated a screening algorithm based on immunohistochemistry (IHC), followed by fluorescence in situ hybridization (FISH) in IHC-positive cases to identify *ALK*-rearranged PTC. IHC and FISH were performed in a cohort of 259 thyroid carcinomas enriched for aggressive variants. IHC was positive in 8 cases, 6 confirmed translocated by FISH (specificity 75%). All 251 IHC-negative cases were FISH negative (sensitivity 100%). Having validated this approach, we performed screening IHC, followed by FISH in IHC-positive cases in an expanded cohort. ALK translocations were identified in 11 of 498 (2.2%) of all consecutive unselected PTCs and 3 of 23 (13%) patients with diffuse sclerosing variant PTCs. No *ALK* translocations were identified in 36 PTCs with distant metastases, 28 poorly differentiated (in-sular) carcinomas, and 20 anaplastic carcinomas. All 14 patients with *ALK* translocations were female (*P* = 0.0425), and translocations occurred at a younger age (mean 38 vs. 48 y, *P* = 0.0289 in unselected patients). *ALK* translocation was an early clonal event present in all neoplastic cells and mutually exclusive with *BRAF*^{V600E} mutation. *ALK* translocation was not associated with aggressive clinicopathologic features (size, stage, metastasis, vascular invasion, extrathyroidal extension, multifocality, risk for recurrence, radioiodine resistance). We conclude that 2.2% of PTCs are *ALK*-translocated and can be identified by screening IHC followed by FISH. *ALK* translocations may be more common in young females and diffuse sclerosing variant PTC but do not connote more aggressive disease.

Key Words: thyroid cancer, papillary thyroid carcinoma, ALK, EML4-ALK

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Papillary thyroid carcinoma (PTC) is the most common endocrine cancer, accounting for 70% to 80% of all new thyroid malignancies.[1] Although PTC typically has an excellent prognosis after surgery with or without adjuvant radioactive iodine (RAI) treatment, some subtypes such as the tall cell, columnar cell, or diffuse sclerosing variants may be more aggressive.[2–5] Further, PTC can also undergo dedifferentiation into the aggressive poorly differentiated thyroid carcinoma (also known as "insular carcinoma") or the extremely aggressive anaplastic thyroid carcinoma, which is almost universally fatal.[3,6,7]

Well-characterized and generally mutually exclusive pathogenic driver mutations, including mutations in *BRAF*, *RAS*, *EIF1AX*, *PPM1D*, and *CHEK2*, and translocations involving *RET*, *BRAF*, *PPARγ*, *NTRK1*, *NTRK3*, *THADA*, and *FGFR2*, have been reported in the great majority of PTCs.[8–16] Recent studies have demonstrated that some PTCs carry an oncogenic fusion gene caused by translocation of the *anaplastic lymphoma kinase* (*ALK*) gene with various partners including *EML4*, *STRN*, *TFG*, and *GTF2IRD1*.[12–16] *ALK* translocations appear to be

mutually exclusive with all other driver mutations and translocations[12–16] and to date have only been identified in PTCs and poorly differentiated and anaplastic carcinomas (presumably arising from dedifferentiation of PTC) but not follicular carcinomas or medullary carcinomas.[12] For example, Kelly et al[12] reported pathogenic *ALK* translocations in 4 of 256 (1.6%) PTCs, 3 of 35 (4%) poorly differentiated carcinomas, and 1 of 24 (4%) of anaplastic carcinomas but in none of 36 follicular or 22 medullary carcinomas and suggested that *ALK* translocations may be more common in aggressive subtypes of PTC.

Crizotinib, a small molecule tyrosine kinase inhibitor rationally designed to target ALK, has proven clinically effective in the treatment of lung adenocarcinomas harboring *ALK* translocations (summarized in Febbo et al[17]). In animal models and in vitro studies,[12] as well as in a single-case report in humans,[18] crizotinib has demonstrated significant antitumor activity in thyroid carcinomas harboring *ALK* translocations. Therefore, the identification and targeting of *ALK* translocations in thyroid carcinoma, so called "drug repurposing," represents a rational therapeutic approach particularly if *ALK* translocations are overrepresented in clinically aggressive thyroid carcinomas.

For these reasons there is a need to better define the true incidence and clinicopathologic features of *ALK*-translocated thyroid carcinomas and to devise an optimal screening strategy to identify this rare molecular event in a common tumor. We therefore sought to develop and implement a screening strategy for *ALK* translocations in thyroid carcinoma on the basis of immunohistochemistry (IHC) with follow-up fluorescence in situ hybridization (FISH) studies only in IHC-positive cases (a model that we and many others use in routine clinical practice in lung cancer[19]). We then used this approach to determine the true incidence and clinicopathologic significance of *ALK* translocations in both unselected PTCs and carcinomas with aggressive behavior including the diffuse sclerosing variant of PTC, PTCs with distant metastases, poorly differentiated (insular) carcinomas, and anaplastic thyroid carcinoma.

MATERIALS AND METHODS

We first sought to validate IHC as a screening tool for *ALK* translocation in both usual PTCs and aggressive thyroid carcinomas. We developed a test cohort comprising tumors from 259 patients. This cohort was enriched for cases with aggressive clinical or pathologic features so that it contained 164 consecutive and truly unselected PTCs (some of which may have been aggressive) and an additional group of 95 aggressive PTCs consisting of another 20 diffuse sclerosing variant PTCs, another 27 PTCs with distant metastases, 28 poorly differentiated (insular) carcinomas, and 20 anaplastic thyroid carcinomas. All cases underwent central pathologic review by an experienced endocrine pathologist (A.J.G.) to confirm the diagnosis. Poorly differentiated (insular) carcinoma was defined by strict application of the Turin criteria,[7] whereas all other tumors including the diffuse sclerosing variant of papillary carcinoma were defined according to the WHO 2004 classification.[3] Six of 28 (21.4%)

poorly differentiated (insular) carcinomas and 5 of 20 anaplastic carcinomas (25%) arose in close association with a component of papillary carcinoma in keeping with the origin from dedifferentiation of a lower-grade tumor. In all cases of poorly differentiated and anaplastic (insular) carcinomas, the PTC component when present was minor (< 10% of the volume of the tumor).

Tissue microarrays (TMAs) were constructed to include two 1 mm cores of formalin-fixed paraffin-embedded tumor tissue from all patients in this cohort. In patients with multiple tumors, the largest tumor was selected. In cases with poor or anaplastic differentiation arising in association with papillary carcinoma, the high-grade (poorly differentiated or anaplastic) components were cored. IHC was performed on TMA sections using the same methods we use to screen lung carcinomas for *ALK* translocation.[19] Briefly, we used a mouse monoclonal antibody to ALK at high concentration (dilution 1:10, clone 5A4; Novocastra, Leica Biosystems, UK). All slides were processed with an automated staining system, the Leica Bond III autostainer (Leica Biosystems) used according to the manufacturer's protocol and with the manufacturer's retrieval solutions. Heat-induced epitope retrieval was performed for 30 minutes in the manufacturer's alkaline retrieval solution ER2 (VBS part no: AR9640). Staining for ALK was interpreted as positive if there was genuine cytoplasmic staining irrespective of whether the staining was patchy or diffuse. All slides were scored by a single pathologist (A.J.G.) who was blinded to all other data (including the results of FISH studies).

All 259 tumors from this cohort also underwent FISH studies. FISH was performed on TMAs using the Vysis LSI ALK Dual-Color Break-Apart Rearrangement Probe (Abbott Molecular). This probe set includes 2 DNA probes, 1 labeled with Spectrum Orange (which is telomeric to the breakpoint of the *ALK* gene at 2p23) and 1 with Spectrum Green (which is centromeric to the breakpoint on the *ALK* gene). Therefore, this probe should identify the presence of any *ALK* rearrangement at the common breakpoint but not the accompanying fusion partner.[19] The FISH studies were interpreted according to the established protocols as applied in lung carcinoma and in parallel with hematoxylin and eosin–stained sections to confirm that only neoplastic cells were assessed. Briefly, FISH signals from 50 malignant cells were assessed, and a case was scored as positive when > 15% of the tumor nuclei showed ≥ 2 signal diameters between the red and green signal (red 3′, green 5′) or if there was an isolated red signal. FISH testing was performed and interpreted by a surgical pathologist with extensive clinical experience in FISH interpretation using this probe (A.C.) who was blinded to all other data (including the results of IHC on the same cohort).

If either IHC or FISH studies could not be interpreted in TMA sections due to insufficient material or ambiguous signals, then the studies were repeated on whole sections. All cases that were positive by IHC or FISH on TMA sections also underwent repeat testing with both IHC and FISH on whole sections.

Cases that were confirmed to be rearranged by FISH studies using the break-apart probe, then underwent second FISH studies on whole sections using the Zytolight SPEC ALK/EML4 TriCheck Probe (ZytoVision, Germany). In addition to the telomeric Spectrum Orange and centromeric Spectrum Green probes, which identify the break-apart in *ALK* at 2p23, this kit also includes a blue fluorochrome, which hybridizes with the *EML4* gene as well as the surrounding region of 2p21. Therefore, in addition to confirming the presence of a translocation involving *ALK* by the separation of orange and green signals, the presence of an additional "split" blue signal colocalizing with the other signals is presumptive evidence that the translocation partner for *ALK* is *EML4*, whereas the absence of an additional blue signal indicates an *ALK* rearrangement without rearrangement of *EML4* and suggests an alternate translocation partner. The TriCheck FISH studies were performed and interpreted using the manufacturer's guidelines by both a scientist (C.S.) and a pathologist (S.O.T.) with extensive clinical experience in FISH interpretation using this probe. At the time of interpretation of the TriCheck probe FISH these observers were not blinded to the IHC and break-apart FISH results.

After screening IHC was demonstrated to be highly sensitive for the presence of *ALK* gene rearrangements, an additional cohort of 334 unselected patients with PTC were identified from the clinical database of the University of Sydney Endocrine Surgery unit. Again the cases were reviewed by an experienced endocrine pathologist (A.J.G.) to confirm the diagnosis and classify according to WHO criteria,[3] and TMAs were constructed containing duplicate 1 mm cores. These cases underwent IHC for ALK and, if positive, then underwent repeat IHC and FISH studies on whole sections with both the Abbot break-apart and Zytolight TriCheck probes.

BRAF[V600E] mutation–specific IHC was performed with clone VE1 (Spring Bioscience, Pleasanton, CA) on
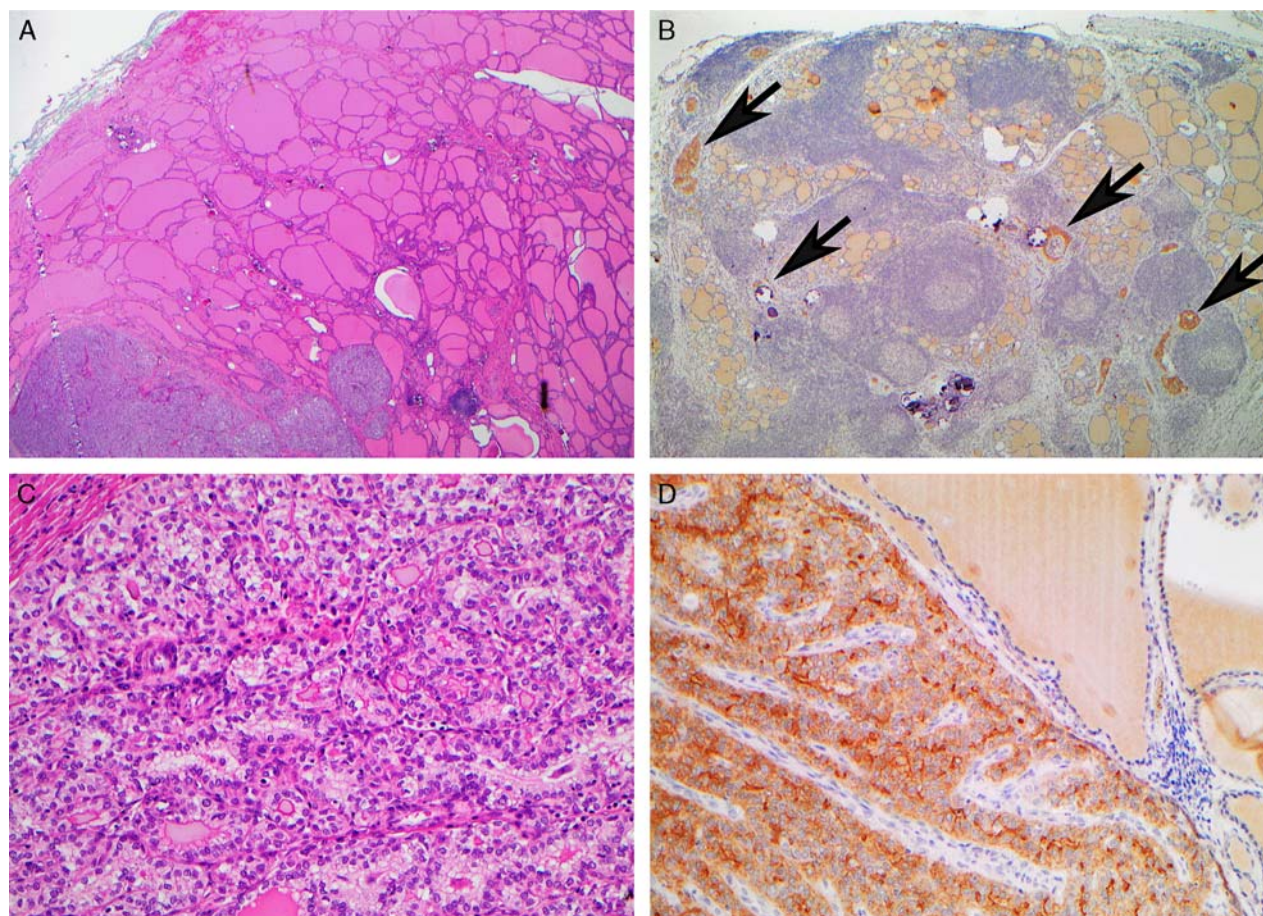
FIGURE 1. A, *ALK*-translocated PTC with a follicular and solid architecture. Although this tumor was considered insufficiently infiltrative to be classified as diffuse sclerosing variant, numerous psammoma bodies are noted in the thyroid away from the main tumor mass, indicating a tendency to lymphovascular invasion. B, ALK IHC on a diffuse sclerosing variant PTC arising in the setting of Hashimoto thyroiditis. ALK highlights small groups of malignant cells (arrows) in lymphatics or the interstitium associated with psammoma bodies. C, *ALK*-translocated PTC with a follicular and solid architecture. D, ALK shows strong diffuse cytoplasmic staining in all neoplastic cells. Although there is some nonspecific staining of colloid, non-neoplastic cells are negative (A and C, hematoxylin and eosin; B and D, ALK IHC).

all cases in TMA format (and repeated on whole sections in ALK translocation–positive cases) using previously described methods, which we have specifically demonstrated to be highly sensitive and specific for the presence of $BRAF^{V600E}$ in our hands.[10] Again $BRAF^{V600E}$ IHC was interpreted by a single pathologist (A.J.G.) who was blinded to all other clinical and pathologic information (including ALK status) at the time of scoring the TMA. $BRAF^{V600E}$ IHC was repeated on whole sections on all ALK IHC-positive and FISH-positive cases.

ALK translocation status was correlated with thyroid cancer survival data collected prospectively within a comprehensive thyroid cancer database previously described.[10,20,21] The rate of thyroid cancer structural recurrence was analyzed on the basis of *ALK*-rearranged status. Univariate analysis was performed using the Fisher exact test for discrete variables and the Student *t* test for continuous variables. Survival outcomes were calculated using the Kaplan-Meier methods and compared using the Log-rank test. The α for significance was set at < 0.05.

RESULTS

From the initial cohort of 259 patients who underwent both FISH and IHC, IHC was positive in 8 (3.2%) cases (Figs. 1, 2). FISH studies using the break-apart probe confirmed the presence of *ALK* gene rearrangements in 6 of these cases (Fig. 3). All 251 cases that were negative for ALK by IHC were confirmed to lack *ALK* rearrangements by FISH studies using the break-apart probe indicating a sensitivity of IHC of 100% in this cohort.

Three of the 6 translocation-positive cases from the group were from the cohort of unselected papillary carcinomas (incidence 1.8%), which incidentally included 3 cases of diffuse sclerosing variant of papillary carcinoma, which were all FISH and IHC negative. The remaining 3 cases that were FISH positive were from the additional group of 20 diffuse sclerosing variant papillary carcinomas. Therefore, the incidence of ALK translocations among all diffuse sclerosing variant PTCs encountered in our institution was 3 of 23 (13%). No IHC-positive or FISH-positive cases were identified in the additional group of 27 PTCs with distant metastases, 28 poorly differentiated (insular) carcinomas, or 20 anaplastic thyroid carcinomas.

Having demonstrated that IHC was highly sensitive albeit not fully specific for ALK translocations, we then performed screening IHC on an additional group of 334 unselected patients with PTC with follow-up FISH studies only in IHC-positive cases. In this second unselected group, 13 cases were positive by IHC, of which 8 were confirmed rearranged by FISH studies (61.5% of IHC-positive cases, 2.4% of this cohort). When the 2 unselected cohorts of patients with PTC were combined, we identified an *ALK* gene rearrangement in a total of 11 of 498 (2.2%) of all unselected patients with PTC.

Additional FISH testing using the Zytolight SPEC ALK/EML4 TriCheck Probe was performed on whole-tissue sections of all tumors that were positive for translocation using the break-apart probe. This confirmed the presence of an *ALK* rearrangement in all 14 cases. In 8 cases (57%), the blue fluorochrome demonstrated an additional signal, which colocalized with the split orange and green signals indicating rearrangement of *EML4* (Fig. 3). In the other 6 *ALK*-rearranged cases, there were split orange and green signals with only 2 blue signals, suggesting that the translocation partner was a gene other than *EML4*.

We noted that in all translocated cases ALK IHC was diffusely positive in all or almost all neoplastic cells (including being present in both primary and metastatic disease) and that FISH studies demonstrated the presence of the translocation in all areas of the tumors that were screened (including areas with weak or patchy IHC staining), indicating that *ALK* gene rearrangements are an early clonal event. All *ALK*-translocated patients demonstrated negative IHC staining for $BRAF^{V600E}$ with mutation-specific IHC, suggesting that *ALK* gene rearrangements and $BRAF^{V600E}$ mutation were mutually exclusive.

The morphology of all 14 *ALK*-translocated cases was specifically reviewed in search of any phenotype-genotype correlations (Figs. 1, 2). In addition to 3 cases of diffuse sclerosing variant PTC, there were 6 unencapsulated follicular variant PTCs, many of which demonstrated areas with a prominent solid architecture, sometimes with relatively subtle nuclear atypia despite an obviously infiltrative growth pattern. There were 2 cases that demonstrated a mixed follicular and papillary architecture but were follicular predominant, again with small areas of solid growth and an obviously infiltrative growth pattern. There was 1 tall cell variant PTC, 1 Hurthle cell variant PTC (again with some solid areas), and 1 Warthin tumor–like variant. Of note, in 5 of 14 cases (36%) the non-neoplastic thyroid demonstrated typical histologic features of Hashimoto thyroiditis.

We then studied the clinical significance of ALK gene rearrangements by comparing translocation status with all available clinical and pathologic data in the cohort of 498 unselected patients with PTC (11 of whom harbored translocations) (summarized in Table 1). Briefly, *ALK*-rearranged PTCs presented at a significantly younger age than nonrearranged cases (mean 38 vs. 48 y, $P = 0.0289$), and there was a statistically significant association with female sex ($P = 0.0425$). In fact, all 14 patients with *ALK* translocations were female. The mean follow-up for translocated cases was 67.3 versus 56.5 months ($P = 0.5854$), and at last follow-up all 11 translocated patients were disease free and had undetectable thyroglobulin levels, whereas in nontranslocated cases there were 50 structural recurrences ($P = 0.3080$), 9 distant metastases ($P = 0.813$), and 105 patients with elevated serum thyroglobulin at last follow-up ($P = 0.0810$). There were no significant differences between *ALK*-rearranged and other PTCs with respect to tumor size, stage, number of lymph nodes resected, number of lymph nodes involved, lymphovascular invasion, extrathyroidal extension, multifocality, number of doses of RAI received or cumulative dose of RAI received.
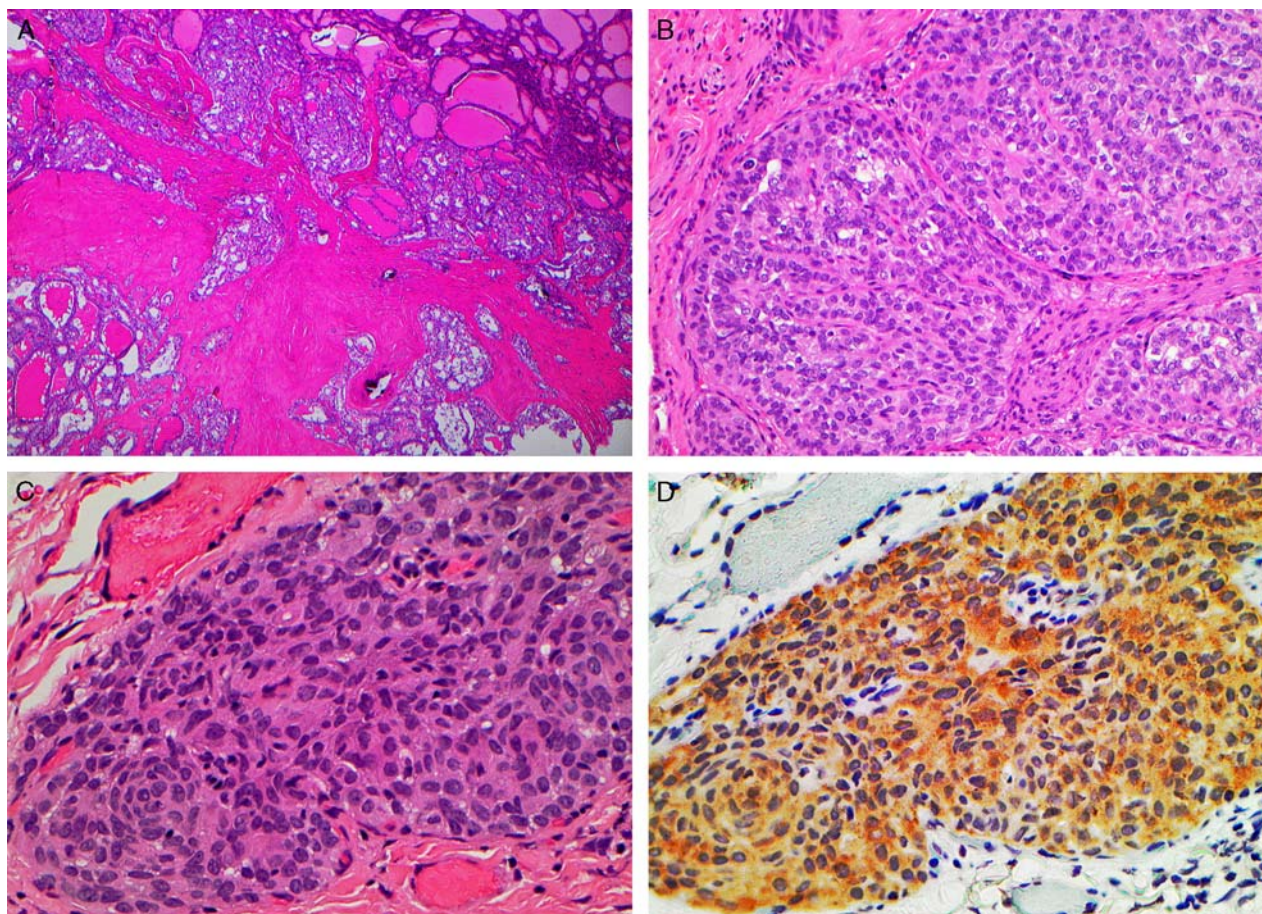
FIGURE 2. A, Unencapsulated follicular variant papillary carcinoma was one of the more common patterns demonstrated by *ALK*-translocated PTC. B, Follicular and solid architectures appeared to be overrepresented in *ALK*-translocated PTC. C, In some areas this *ALK*-translocated carcinoma demonstrated a solid and almost morula architecture reminiscent of a solid cell rest. D, Serial sections of this area demonstrates diffuse strong positive staining for ALK (A–C, hematoxylin and eosin; D, ALK IHC).

DISCUSSION

PTC is inherently an indolent malignancy with a 10-year survival of > 93%.[1,2,3,22,23] However, recurrence and metastases still occur in 10% to 15% of patients, two thirds of whom will eventually develop RAI refractory disease.[23–26] Therefore, there is still a need to develop new
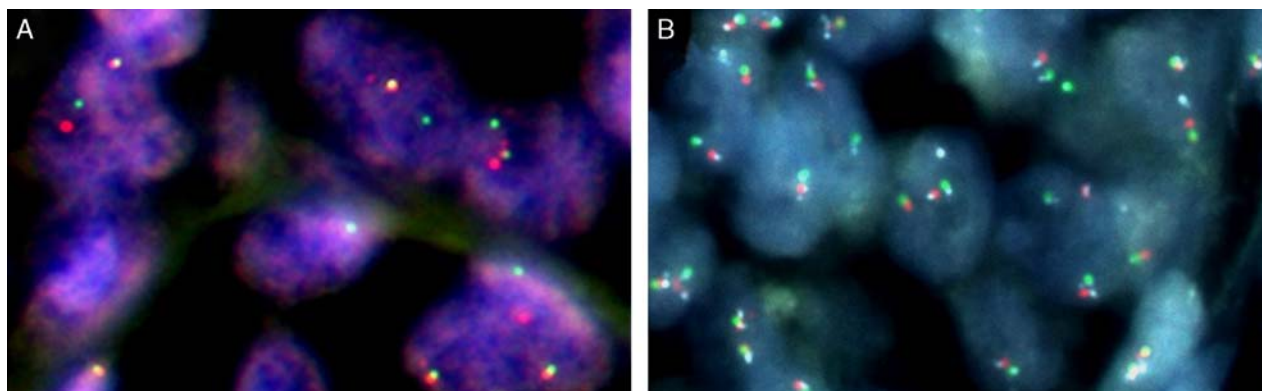
FIGURE 3. FISH studies from *ALK*-translocated cases. A, Using the break-apart probe *ALK*-rearranged PTC with >15% of tumor nuclei showing splitting of the orange (3′) and green (5′) signals. B, Using the Tricheck probe, the splitting of orange and green signals is still evident; however, an extra "split" blue signal is now present and colocalizes with the split orange and green signals, suggesting that the translocation partner is EML4.

TABLE 1. Clinical and Pathologic Features of ALK-rearranged Versus Non–ALK-rearranged PTC in Unselected Patients

	ALK-rearranged (n = 11)	Non–ALK-rearranged (n = 487)	P
Sex			
Male	0	123	
Female	11	364	**0.0425***
Age of onset (mean [range]) (y)	38 (13-68)	48 (10-88)	**0.0289†**
Tumor size (mean [range]) (mm)	25 (7-67)	22 (0.9-112)	0.5005‡
T stage			
T1-2	5	294	0.3523‡
T3-4	6	185	
N stage			
N0	8	292	0.5415‡
N1	3	187	
No. nodes removed at initial surgery (mean)	6.3	7.4	0.7456‡
No. positive nodes at first surgery (mean)	1.8	2	0.8838‡
Lymphovascular invasion			
Present	3	140	
Absent	8	338	0.5920*
Extrathyroidal extension			
Present	3	174	
Absent	8	316	0.4100*
Multifocality			
Present	4	213	
Absent	7	268	0.4200*
No. RAI given (average)	1.1	1.1	0.8468‡
Cumulative RAI dose (average)	4.8	5.4	0.6763‡
Structural recurrence			
Present	0	50	0.3080*
Absent	11	437	
Thyroglobulin at last follow-up			
Positive	0	105	0.0810*
Negative	10	363	
Distant metastasis			
Present	0	9	
Absent	11	469	0.8130*
Follow-up time (average) (mo)	67.3	56.5	0.5854‡
Progression-free survival (median) (mo)	No recurrence	226	0.2340§

*Fisher exact test (1 tailed).
†Unpaired *t* test (1 tailed).
‡Unpaired *t* test (2 tailed).
§Log-rank test.
The values in bold are *P*-values statistically significant.

treatments for patients with resistant disease. *ALK* gene rearrangements have been reported to show all the characteristics of driver mutations[12,16] and to be mutually exclusive with all other driver mutations in PTC,[12,13,14,15,16] and therefore represent an attractive potential therapeutic target. However, before this targeted therapy can be investigated in an appropriate clinical trial, there remains the very real difficulty of how to identify these rare gene rearrangements in a common malignancy in a cost-effective and practical manner.

Although there is widespread acceptance that FISH studies are the gold standard for identifying *ALK* translocations in lung cancer,[17,27] these studies are expensive and, because of the subtlety of intrachromosomal inversion events, potentially difficult to interpret in the routine clinical setting.[19] Therefore, one increasingly widespread approach in lung cancer is to perform screening IHC with next-generation highly sensitive ALK antibodies (eg, clones 5A4 or D5F3) and then perform confirmatory FISH

studies only in IHC-positive cases.[19,28–31] This approach has been endorsed by professional bodies provided IHC has been validated in the local setting[32] and has the significant advantage of low cost and rapid turnaround time.[19]

In this study, we confirm that the approach of performing screening IHC followed by FISH studies only in IHC-positive cases is valid in PTC and aggressive tumors presumably arising from PTC (anaplastic and poorly differentiated "insular" carcinoma). In fact, from the initial cohort that underwent FISH and IHC, all 251 cases that were IHC negative lacked *ALK* rearrangements on FISH studies, whereas 6 of the 8 IHC-positive cases were confirmed to be translocated by FISH. We caution that if major management decisions may potentially be made on the basis of IHC findings, there is a need to validate this approach with the specific antibodies and conditions present in individual laboratories. However, as we used the same protocols for this study that we have validated and now routinely use in lung cancer,[19] it is likely that if an

individual laboratory has validated an IHC-based screening strategy followed up by FISH studies in lung cancer then the same protocol is likely to be valid in thyroid carcinoma.

Using this algorithm we demonstrate that at least 11 of 498 (2.2%) unselected PTCs harbor gene rearrangements involving *ALK*. Because initial screening IHC was performed only on TMA sections, it is possible that cases with subclonal *ALK* gene rearrangements would not be identified by our approach; however, given that *ALK* gene rearrangements were early clonal events and present in all neoplastic cells, we think this would be a rare phenomenon if it occurs at all. Our incidence of *ALK* rearrangements is similar but slightly greater than that reported by Kelly et al,[12] who found *ALK* gene rearrangements in 4 of 256 (1.6%) of all PTCs, McFadden et al,[13] who found rearrangements in 2 of 126 (1.6%) follicular variant PTCs, and the Cancer Genome Atlas study, which found rearrangements in 4 of 484 PTCs (0.8%).[16]

Before this study, the rearrangement partners for *ALK* have been reported in 28 patients.[12–16,18,33] In 15 cases (54%) the translocation partner has been *EML4*, in 11 cases *STRN*, and in 1 case each *TFG* and *GTF2IRD1*. In addition to the usual break-apart probe, in this study we also performed FISH with the Zytolight SPEC ALK/EML4 TriCheck Probe, which includes a blue fluorochrome that hybridizes with a 1.8 Mbp region of chromosome 2p21 including the entire *EML4* gene as well as the surrounding region from D2S1481 to D2S1494E. In 8 cases (57%) this blue fluorochrome produced a split signal, which colocalized with split orange and green signals, suggesting the presence of an *ALK-EML4* inversion. In the other 6 translocated cases, there were split orange and green signals with 2 intact blue signals, suggesting the presence of *ALK* rearrangement without involvement of *EML4*. We caution that this aspect of the tricheck probe has not been validated by an independent method, and it is possible that other translocation partners may be involved; however, our findings are strong presumptive evidence that the translocation partner for *ALK* was *EML4* in 57% of cases and another gene(s) in 43% of cases.

This is the first study to systematically investigate the clinicopathologic features of *ALK*-rearranged PTC. It is noteworthy that all 14 *ALK*-rearranged PTCs in our study occurred in females individual ($P = 0.0425$) and that *ALK*-rearranged PTCs presented in patients 10 years younger than those with other tumors—another finding that reached statistical significance ($P = 0.0289$). It has previously been suggested that *ALK* rearrangements are more common in aggressive disease.[12] However, this is not supported by our data. We found no *ALK* rearrangements in 36 PTCs with distant metastases (9 from the unselected cohort and 27 additional cases screened), 28 poorly differentiated (insular) carcinomas, and 20 anaplastic thyroid carcinomas. Further, none of 11 *ALK*-rearranged PTCs from the unselected cohort recurred (either structurally or with elevated serum thyroglobulin), and there were no significant differences between *ALK*-rearranged and other PTCs with respect to tumor size, stage, number of lymph nodes resected, number of lymph nodes involved, lymphovascular invasion, extrathyroidal extension, and multifocality. Further, given that there was no difference in the number or cumulative doses of RAI received, it is unlikely that *ALK*-rearranged PTC is more likely to be RAI resistant.

When we specifically reviewed the histology of *ALK*-rearranged PTCs in search of genotype-phenotype correlations, we did note that 6 cases were unencapsulated follicular variant PTCs, commonly with areas of solid growth (Figs. 1, 2), and 2 cases included areas with a follicular architecture but demonstrated papillary areas. Our findings suggest that an infiltrative follicular architecture, perhaps with areas of solid growth, may be overrepresented in *ALK*-translocated PTCs. However, other morphologies including 1 tall cell variant, 1 Hurthle cell variant, and 1 Warthin tumor–like variant were noted, and this morphology is unlikely to be specific enough to help identify these cases without IHC or FISH studies.

It is interesting that *ALK* gene rearrangement was identified in 3 of 23 (13%) diffuse sclerosing variants of papillary carcinoma—a subtype of PTC that is itself more common in young female individuals (the same demographic as *ALK*-translocated PTC) and has been reported to have a 25% incidence of lung metastasis.[3] The diffuse sclerosing variant of PTC has been reported to have a very low rate of $BRAF^{V600E}$ mutation,[34] and indeed in our cohort, 5 of 23 (22%) harbored this mutation versus 62.4% from our unselected cohort. Although the number of cases is small and our results should be interpreted with caution, given that $BRAF^{V600E}$ mutation and *ALK* rearrangement are mutually exclusive, *ALK* rearrangement may be a relatively frequent driver mutation in diffuse sclerosing variant PTCs. Our finding that 5 of 15 (36%) of PTCs with *ALK* rearrangement were associated with histologic evidence of Hashimoto thyroiditis is interesting given that Hashimoto thyroiditis has previously been associated with another translocation (*RET-PTC*).[35] However, this potential link requires further investigation before any conclusions can be made.

In conclusion, 2.2% of PTCs harbor translocation of the *ALK* gene, and these cases can be effectively identified using screening IHC followed by FISH. ALK gene rearrangement occurs more commonly in female individuals and at a younger age and is mutually exclusive with $BRAF^{V600E}$. *ALK* gene rearrangement may occur more commonly in diffuse sclerosing variant PTC but does not appear to predict more aggressive behavior.

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