Identification of a Recurrent *LMO7-BRAF* Fusion in Papillary Thyroid Carcinoma

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Background: The *BRAF*^{V600E} mutation is the most common driver in papillary thyroid carcinoma (PTC) tumors. In recent years, gene fusions have also been recognized as important drivers of cancer in PTC. Previous studies have suggested that thyroid tumors with fusion genes frequently display an aggressive course. These observations prompted further exploration of gene fusions in PTC tumors. The aim was to search for previously unrecognized gene fusions using thyroid tissue samples from PTC patients.

Methods: Gene fusions were analyzed in RNA sequencing data obtained from 12 PTC tumors and paired unaffected thyroid tissue samples. Candidate fusions were further filtered and validated using reverse transcriptase polymerase chain reaction, Sanger sequencing, and fluorescence *in situ* hybridization. An Ohio cohort of 148 PTC tumor samples was screened for a *LMO7-BRAF* fusion and the *BRAF*^{V600E} mutation. Functional assays were performed to assess the *LMO7-BRAF* fusion.

Results: Two coding fusions (*CCDC6-RET* and *LMO7-BRAF*) were found in one tumor sample each. The novel *LMO7-BRAF* fusion was validated by reverse transcriptase polymerase chain reaction and fluorescence *in situ* hybridization. The *LMO7-BRAF* fusion was a recurrent somatic alteration with a frequency of 2.0% (3/148) in PTC tumors, while the *BRAF*^{V600E} point mutation was found in 63.5% (94/148) of tumors. Enforced expression of *LMO7-BRAF* fusion protein stimulated endogenous ERK1/2 phosphorylation and promoted anchorage independent cell growth to an extent similar to *BRAF*^{V600E}.

Conclusions: A novel fusion gene, *LMO7-BRAF*, was identified in PTC tumors. The results indicate that the *LMO7-BRAF* fusion behaves as an oncogenic alteration. This observation expands the spectrum of fusion genes involving kinases in thyroid cancer.

Keywords: papillary thyroid carcinoma, fusion gene, *LMO7-BRAF*, *BRAF*^{V600E}

Introduction

THYROID CANCER IS ONE of the few cancers that has increased in incidence in recent years (1,2). A recent study revealed that the overall incidence of thyroid cancer in the United States increased by 3% annually from 1974 to 2013. This may be a true increase, or may be due to overdiagnosis of papillary microcarcinomas (2,3). It is estimated that 56,870 individuals in the United States will be diagnosed with thyroid cancer in 2017 (4). Most thyroid cancers are classified as non-medullary thyroid carcinoma (NMTC). Papillary thyroid carcinoma (PTC) is the main form of NMTC, accounting for approximately 80% of all thyroid cancers. Somatic mutations play an important role in the development of PTC. About two-thirds of PTC tumors harbor somatic genetic alterations in the effec-

tors of the mitogen-activated protein kinase (MAPK) signaling cascade. Overall, two types of genetic alterations in the MAPK pathway have been described in PTC: point mutations (e.g., in the B-Raf proto-oncogene, serine/threonine kinase [*BRAF*], and the rat sarcoma virus oncogene [*RAS*] genes), and chromosomal translocations involving, among many others, *CCDC6-RET* (formerly *RET/PTC1*), *NCOA4-RET* (formerly *RET/PTC3*), or *TPM3-NTRK1* (5,6). *BRAF*^{V600E} has been described as the most common point mutation in PTC, being mutually exclusive with the *RET/PTC1* rearrangement (7). However, their co-occurrence was recently found in nearly 20% of PTC tumors (8). Innovative sequencing technologies (next-generation sequencing [NGS]) have revolutionized cancer research, and several previously unknown fusion genes have been identified in PTC tumors, such as *AKAP9-BRAF* and *WNK1-B4GALNT3*

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(9,10). It has been reported that fusion genes make up 10% of all driver mutations in PTC, and the tumors with fusion genes usually present a more aggressive course (11).

This study describes the identification by NGS RNA sequencing (RNA-Seq) of a novel fusion gene, *LMO7-BRAF*, in which the 5' portion of the LIM domain 7 (*LMO7*) gene fused with the 3' portion of the *BRAF* gene. The *LMO7-BRAF* fusion is a recurrent abnormality in PTC tumors, with a frequency of 2.0%.

Methods

This study was approved by the Cancer Institutional Review Boards at the Ohio State University Medical Center. All subjects gave written informed consent for participation.

Tissue samples and cell lines

Thyroid tumor tissue samples ($n=148$) were obtained from PTC patients undergoing surgical resection. Twelve tumor and noncancerous tissue pairs were studied by RNA-Seq (12). The samples were snap-frozen in liquid nitrogen and stored at -80°C . Clinical data and information regarding the specimens are shown in Table 1. NIH3T3 and HEK293 cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 .

RNA isolation and quality assessment

Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The purity of extracted RNA was measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc.), and the concentration was assessed with Qubit 2.0 Fluorometer (Agilent Technologies) using an RNA HS Assay Kit. Samples with a RNA Integrity Number (RIN) >4 as assessed by a BioAnalyzer (Agilent) with no visible sign of genomic DNA contamination from the HS Nanochip tracings were used for total RNA library generation.

Preparation of strand-specific RNA-Seq libraries and RNA-Seq

The RNA-Seq procedures and the clinical information about the 12 paired PTC tumor and noncancerous tissue samples have been published previously (12). Briefly, the Illumina TruSeq Stranded Total RNA Sample Prep Kit with Ribo-Zero Gold (catalog #RS-122-2201) was used. The protocol consisted of random primer and stranded RNA extraction, RNA fragmentation, reverse transcription, and 100 bp paired-end sequencing using the Illumina HiSeq 2500 system. Pre-alignment data QC was assessed with FastQC. Post-alignment data quality was assessed with an in-house quality control pipeline/database for the RNA-Seq database (13). The RNA-Seq data have been deposited in the Gene Expression Omnibus (GEO) repository and are accessible through the GEO accession number GSE83520.

Fusion transcript identification

To identify fusion transcripts specific for PTC, the fusion detection program Fusion Catcher was used (14), using the "mildly_sensitive" option, with hg19 sequence reference from UCSC and Ensembl release 69 annotation. To enrich for true-positive fusion transcripts specifically expressed in PTC cells,

TABLE 1. CLINICOPATHOLOGIC CHARACTERISTICS OF THE OHIO PTC COHORT

Characteristics	Case number
Sex	
Female	113
Male	35
Age at diagnosis	
Median (range)	43 (16–85)
Age <45 years	79
Age ≥ 45 years	69
Histo-subtype	
Classic PTC	123
MicroPTC	6
Follicular variant	8
Oncocytic variant	5
Tall-cell variant	2
Other variants ^a	4
N stage	
N0	80
N1	67
Unknown	1
T stage	
T1	54
T2	42
T3	48
T4	2
Unknown	2
Tumor size (cm)	
Median (range)	2.3 (0.4–7.5)
Tumor focality	
Unifocal	77
Multifocal	71
M stage	
M0	147
M1	0
Mx ^b	1
BRAF ^{V600E}	
No	54
Yes	94

^aOther variant: columnar cell, Hürthle cell, solid, or Warthin-like (one case of each).

^bMx, distant metastasis cannot be evaluated.

PTC, papillary thyroid carcinoma.

several filtering steps of the initial fusion breakpoint candidates were performed. Candidate fusions between genes were called when they were separated by a distance $>100,000$ bp and supported by at least two reads spanning the fusion point.

To evaluate the authenticity of the fusion gene candidates further, each fusion candidate was examined by using FuSpot, a program for the visualization of candidate fusion points using reads from the samples in which the candidate fusions were identified. A minimum alignment percentage of $\geq 95\%$ was used, and candidates were selected with reads that aligned to the breakpoint region.

Validation and screening of fusion transcript breakpoints by reverse transcriptase polymerase chain reaction and Sanger sequencing

Two fusion transcript candidates (*CCDC6-RET* and *LMO7-BRAF*) were validated with reverse transcriptase

polymerase chain reaction (RT-PCR) in the original index samples. These two fusions were further screened in an Ohio cohort of PTC tumor tissue samples ($n=148$). Total RNA was first treated with DNase-1 and then reverse transcribed to cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Primers were designed to the fusion transcript breakpoint sequences using the Primer3 web application (15). The primer sequences used for the *LMO7-BRAF* fusion were: *LMO7*-forward, 5'-AGC ACA AAG CAA CCC GTA CT; *BRAF*-reverse, 5'-CAC CAC GAA ATC CTT GGT CT. Briefly, RT-PCR was performed using the high-capacity reverse transcription kit according to protocol (Applied Biosystems by Life Technologies). From 50 ng of starting cDNA template, a PCR protocol was initiated with 5 min of HotStarTaq DNA polymerase activation at 95°C, followed by 30 thermal cycles of denaturation for 30 s at 95°C, primer annealing for 1 min at optimal primer melting temperatures, and extension for 1 min at 72°C. To ensure specific amplification of the breakpoint sequences, PCR products positive for the *LMO7-BRAF* fusion were Sanger sequenced using the ABI 3730 DNA Analyzer (Applied Biosystems). Two sets of primers were used for PCR and sequencing: Seq-Forward1, 5'-CTC TCT CCC TCC CTT GTC CT, and Seq-Reverse1, 5'-CTT CCT TTC TCG CTG AGG TC; Seq-Forward2, 5'-TAC TGG CTG GGA AGA AAA GC, and Seq-Reverse2, 5'-CCA CAA AAT GGA TCC AGA CA. The resulting sequences were analyzed using Sequencing Analysis v5.3.1 software.

Fluorescence in situ hybridization analysis

Dual-color fluorescence *in situ* hybridization (FISH) was performed on PTC tumor touch imprints (TTIs) from fresh-frozen tumor pieces of the index case, as previously described (16). The FISH probes were provided by Obio LLC (GSP Laboratory; www.gsplab.co.jp) and are illustrated in Figure 2A. The imprints were done by gently touching the tissue onto the clean, charged slide and immediately fixing it in Carnoy's fixative for 20 min at 4°C. FISH was done according to the manufacturer's recommendations, except prior to hybridization, slides were pretreated with pepsin and postfix solution. Co-denaturation of probe and sample was done on HyBrite (Vysis, Inc.) for 5 min at 75°C. Hybridization was carried out overnight at 37°C, and slides were washed in $2 \times \text{SSC}/0.1\%$ NP-40 for 2 min at 73°C. After counterstaining with DAPI, the signals were viewed using a fluorescence microscope (Zeiss Axioscope 40) equipped with appropriate filters and analyzed with Applied Imaging System. At least 50 cells from five different prints were analyzed.

Expression constructs and DNA transfection

Expression construct of the *pcDNA3-LMO7-BRAF* fusion was synthesized, subcloned, and sequenced by Invitrogen GeneArt (Thermo Fisher Scientific). Plasmids *pcDNA3-BRAF*(wt) and *pcDNA3-BRAF*^{V600E} were constructed in the authors' laboratory. The coding sequences of these constructs were confirmed by Sanger sequencing. Transfections of NIH3T3 and HEK293 cells were performed by the Lipofectamine 2000 method, as suggested by the manufacturer (Invitrogen).

Immunoblotting

Cells were washed in phosphate-buffered saline and lysed in RIPA buffer (Thermo Fisher Scientific). Equal amounts of cell extracts were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Following separation, the proteins were transferred to nitrocellulose membranes and blotted with the appropriate primary and secondary antibodies. Antibodies against ERK (cat. #4695; Cell Signaling), phospho-ERK (cat. #4370; Cell Signaling), and ACTIN (ACTN05; Thermo Fisher Scientific) were used.

Soft agar colony formation assay

NIH3T3 cells were seeded into 60 mm plates and transfected with the indicated plasmid for 48 h. The cells were then trypsinized and plated onto a 96-well plate with soft agar by using a Cytoselect 96-well soft agar cell transformation assay kit (Cell Biolab). The assay was carried out according to the manufacturer's protocol.

Detection of the *BRAF*^{V600E} mutation

Genomic DNA was extracted from the tumor tissue samples, and a fragment spanning codon 600 of the *BRAF* gene was amplified by PCR. The PCR products were analyzed by SNaPshot, as described previously. The sequences of the PCR primers are: *BRAF*-forward TGC TTG CTC TGA TAG GAA AAT G; *BRAF*-reverse, CCA CAA AAT GGA TCC AGA CA; and *BRAF*-extension, AGG TGA TTT TGG TCT AGC TAC AG.

Searching for the *LMO7-BRAF* fusion gene in gene fusion databases

Publicly available fusion gene databases were searched, including The Cancer Genome Atlas (TCGA) fusion gene database (www.tumorfusions.org/) (5), ChiTaRS 3.1 (http://chitars.md.biu.ac.il/index.html) (17), and FARE-CAFÉ (18).

Results

Identification of *LMO7-BRAF* fusion transcript in PTC from RNA-Seq data

Fusion transcript analyses of the RNA-Seq data resulted in two fusion transcripts that retained open reading frames (ORFs). One of them (*CCDC6-RET*, also known as *RET/PTC1*) has been reported previously as a frequent *RET* rearrangement in PTC (19). Another fusion transcript, *LMO7-BRAF*, has not been reported in the literature. These two coding fusions in the index samples were validated by RT-PCR. Confirmation of the *LMO-BRAF* fusion was obtained by RT-PCR with primers located in exon 6 of *LMO7* (NM_001306080.1) and exon 9 of *BRAF*, which yielded the expected product (Fig. 1A). Sanger sequences of the *LMO7-BRAF* fusion RT-PCR products confirmed the breakpoints between the *LMO7* and *BRAF* genes, and determined the intact exon-exon boundary between *LMO7* and *BRAF* (Fig. 1B). Using Sanger sequencing, it was further confirmed that exons 1–6 of *LMO7* transcript variant 3 (NM_001306080.1) were present in the fusion gene. The ORF of *LMO7* (exons 1–6) theoretically encodes a truncated protein with 154 amino acids, while the ORF of *BRAF* (exons 9–18) theoretically encodes the partner gene with kinase potential. Multiple RT-PCR reactions were performed, and no

FIG. 1. Validation of and screening for the *LMO7-BRAF* fusion in papillary thyroid carcinoma (PTC) tumors. (A) Totally, 148 tumor samples were screened; 14 samples are shown in this gel picture. Reverse transcriptase polymerase chain reaction (PCR) was performed using the forward primer in *LMO7* exon 6 and the reverse primer in *BRAF* exon 9. Samples #2, #10, and #13 are positive for the *LMO7-BRAF* fusion. Sample #10 is the index sample in which the *LMO7-BRAF* fusion was originally identified with RNA sequencing (RNA-Seq) data. The *BRAF*^{V600E} mutation was screened in the same samples, and the results are shown. +, positive; –, negative. *GAPDH* was used as a control. M, molecular marker; (–), negative control for PCR. (B) Sanger sequencing chromatograph of the *LMO7-BRAF* fusion in sample #10. The arrow shows the breakpoint of the fusion between *LMO7* (NM_001306080.1, end of exon 6) and *BRAF* (NM_004333.4, start of exon 9).

splice variants of the fusion transcript were detected in the tumor cDNAs.

Further confirmation of the *LMO7-BRAF* fusion was obtained by dual-color FISH with probes corresponding to the *BRAF* and *LMO7* genes. The details of the probes are provided in Figure 2A. A FISH image of the tumor touch imprints of the index case (sample #10) is shown in Figure 2B. The yellow-colored signals indicate fusion of the red (indicating *LMO7*) and green (indicating *BRAF*) signals. The majority of the cells had one fusion, two red, two green pattern, indicating fusion of *LMO7* and *BRAF*.

We searched publicly available fusion gene databases, such as TCGA fusion gene database (www.tumorfusions.org/) (5), ChiTaRS 3.1 (17), and FARE-CAFÉ (18). The *LMO7-BRAF* fusion was not found, although the *BRAF* fusion partner (*BRAF* exons 9–18) was reported in the *AKAP9-BRAF* fusion in PTC and appeared as a common fusion partner in solid tumors (9,20).

Ectopic expression of LMO7-BRAF increases ERK phosphorylation and promotes anchorage-independent cell growth

To elucidate the expression and kinase activating potential of the *LMO7-BRAF* fusion protein, HEK293 cells were transiently transfected with *LMO7-BRAF*, *BRAF*^{V600E}, *BRAF*(wt), and empty vector constructs. The endogenous ERK phosphorylation level was measured by immunoblotting (Fig. 3A). The *LMO7-BRAF* fusion protein stimulated endogenous ERK1/2 phosphorylation to an extent similar to *BRAF*^{V600E} in transfected cells, whereas overexpression of *BRAF*(wt) had no discernible effect in comparison with cells transfected with empty vector. To determine if *LMO7-BRAF* induces onco-

genic transformation of NIH3T3 cells, soft agar colony formation assays were performed. Ectopic expression of *LMO7-BRAF* or *BRAF*^{V600E} revealed significantly increased anchorage-independent cell growth when compared with *BRAF*(wt) or vector control (Fig. 3B).

LMO7-BRAF is a recurrent fusion in PTC

The *LMO7-BRAF* fusion was assessed in an Ohio cohort of PTC patients ($n = 148$) using RT-PCR, and three tumors were detected (samples #2, #10, and #13) with the fusion, as shown in the gel image of 14 samples in Figure 1A. It was noticed that the RT-PCR band intensities varied, with sample #10 (the index sample) showing the strongest band intensity, while samples #2 and #13 had faint bands (Fig. 1A). The RT-PCR reactions were repeated twice, and similar patterns of band intensities were observed in these three samples. The RT-PCR products of samples #2 and #13 were Sanger sequenced, and the fusion breakpoints were confirmed as the same as in sample #10. These observations indicated that the *LMO7-BRAF* fusion is a recurrent fusion in 3/148 (2.0%) PTC tumors.

BRAF^{V600E} mutation detection

The *BRAF*^{V600E} mutation was screened in the same sample set, and 94/148 (63.5%) tumors were found carrying this point mutation. Notably, among the three tumor samples positive for the *LMO7-BRAF* fusion, two samples (samples #2 and #13) were also positive for the *BRAF*^{V600E} point mutation (Fig. 1A). These two samples showed weaker band intensities for the *LMO7-BRAF* fusion in the agarose gel. The clinical information pertaining to these three cases is

FIG. 2. Detection of *LMO7-BRAF* with fluorescence *in situ* hybridization (FISH). (A) Diagram of the FISH probes (kindly provided by Obio). The dual-colored FISH probes cover the fusion partners of the *LMO7* and the *BRAF* genes. The genomic coordinates of the fusion point in the *LMO7-BRAF* fusion transcript were obtained from UCSC genome browser (hg19). *LMO7*: chr13:76370885, forward strand; *BRAF*: chr7:140487384, reverse strand. (B) FISH images were obtained with tumor touch imprints from sample #10. Three types of colored signals are shown: red for the *LMO7* gene, green for the *BRAF* gene, and yellow for the fusion between the *LMO7* and the *BRAF* genes.

provided in Table 2. Unique clinicopathologic characteristics were not observed among these three cases.

Discussion

Chromosomal rearrangements are common genetic abnormalities involved in the initiation of cancer development. These rearrangements result in the disruption of genetic material, which can lead to the expression of oncogenic fusion proteins or the disruption of processes involved in tumor suppression (21–23). Gene fusions have been recognized as important drivers of cancer in a wide array of cancer types, including thyroid cancer (20,24). Recurrent gene fusions that drive oncogenesis are frequent in thyroid cancers, with *RET* fusions detected in ~35% of PTC in North America (19). Among *RET* fusions, *CCDC6-RET* (*RET/PTC1*) is a frequently found fusion in PTC (19,25).

RNA-Seq was performed in 12 paired PTC samples, and *CCDC6-RET* and *LMO7-BRAF* fusions were found in one

tumor each. Both fusions were validated by RT-PCR and Sanger sequencing of the fusion regions. Since the *CCDC6-RET* fusion has been characterized before, it was not pursued further. The *LMO7-BRAF* fusion is a novel gene rearrangement, which was not reported in the gene fusion databases such as TCGA fusion gene database (5,26), ChiTaRS 3.1 (17), and FARE-CAFÉ (18). The *LMO7* gene encodes a protein containing a calponin homology domain, a PDZ domain, and a LIM domain, and may be involved in protein–protein interactions. The *BRAF* rearrangements in which the *BRAF* kinase domain is fused to a variety of 5′ partners, have been reported in several solid tumor types as well as in PTC (26,27). For instance, a recurrent fusion gene *AKAP9-BRAF* was found in radiation-exposed PTCs and results from an in-frame fusion of the exons 1–8 of the a-kinase anchor protein 9 (*AKAP9*) gene to exons 9–18 of the *BRAF* gene (9). In TCGA fusion gene database (www.tumorfusions.org/), among the 513 thyroid carcinoma samples, there are nine in-frame fusions identified, with seven unique 5′ fusion partners fused

FIG. 3. *LMO7-BRAF* fusion induces MAPK activation and promotes anchorage-independent cell growth. (A) Immunoblotting of lysates from HEK293T cells transfected with pcDNA3 control vector, or plasmids encoding *BRAF*(wt), *BRAF*^{V600E}, and *LMO7-BRAF* fusion. The primary antibodies to phospho-ERK1/2 (pERK), total ERK (ERK), and actin (ACTIN) were applied. (B) Soft agar transformation assay of NIH 3T3 cells. The bar graph displays the relative anchorage independent cell growth analyzed by fluorimetric assays. All values are presented as mean ± standard deviation of assays performed in triplicate. **p* < 0.05, two-tailed Student's *t*-test.

TABLE 2. CLINICAL CHARACTERISTICS OF THE THREE PTC CASES WITH LMO7-BRAF FUSION

Sample ID	Age (years)	Sex	T stage	N stage	M stage	Histo-subtype	Tumor diameter (cm)	Multifocality	Lymphovascular invasion	CLT	BRAF ^{V600E}
Sample 10 ^a	57	Female	T1	N0	M0	Classic PTC	1.3	Yes	No	No	No
Sample 2	22	Female	T3	N0	M0	Classic PTC	1.4	Yes	No	Yes	Yes
Sample 13	68	Female	T3	N0	M0	Oncocytic PTC	4.3	No	No	No	Yes

^aIndex case, used in RNA sequencing.

CLT, chronic lymphocytic thyroiditis.

with *BRAF* with the removal of at least the first eight exons of *BRAF*. The fusion protein of *BRAF* (exons 9–18) lacks the autoinhibitory N-terminal portion of *BRAF*, but contains the protein kinase domain. Interestingly, the removal of at least the first eight exons of *BRAF* appears in multiple cancer types, and has previously been shown to promote *BRAF* dimerization, independent of activated *RAS* or other mechanisms of *BRAF* dimerization (20,28–30). Fusions containing the *BRAF* partner (exons 9–18) have been reported in PTC, such as *PICALM-BRAF*, *NFYA-BRAF*, and *SND1-BRAF* (31). The *LMO7-BRAF* fusion described in this study has the same *BRAF* partner as these reported fusions. Functional assays with ectopic expression of *LMO7-BRAF* fusion showed elevated kinase activity and increased anchorage-independent cell growth of NIH3T3 cells to an extent similar to *BRAF*^{V600E}, indicating the *LMO7-BRAF* rearrangement is an oncogenic fusion (32). All these together suggest that *BRAF* fusion may represent an alternative mechanism of *BRAF* activation in PTC tumors.

The *BRAF*^{V600E} point mutation was further screened in the same panel of PTC tumors. Notably, two samples positive for the *LMO7-BRAF* fusion were also positive for the *BRAF*^{V600E} mutation. The co-existence of the *LMO7-BRAF* fusion and the *BRAF*^{V600E} point mutation in two tumors is consistent with the notion of unanticipated complexities in the patterns of somatic alterations in cancers (8,33,34). On the other hand, the *BRAF*^{V600E} mutation was observed in 94/148 (63.5%) tumors, which is consistent with the notion that the *BRAF*^{V600E} mutation is the most common somatic change in PTC. It was noticed that two samples (#2 and #13) showed weaker band intensities for the *LMO7-BRAF* fusion in RT-PCR gel, which perhaps reflects a sub-clone in each of these two cases. The patient listed as sample #2 was diagnosed as classic PTC at 22 years of age and underwent surgery and radioiodine treatment. The patient listed as sample #13 was diagnosed with an oncocytic PTC at 68 years of age with no other clinical information available. In a screen for *BRAF* gene fusions in 20,573 solid tumors, Ross *et al.* identified *BRAF* fusions involving the intact in-frame *BRAF* kinase domain in 55 (0.3%) cases across 12 different tumor types, including three cases (3/294; 1.0%) of thyroid cancer. The authors identified a single case of metastatic non-Spitzoid melanoma with both an *AGAP3-BRAF* fusion and a *BRAF*^{V600E} mutation (20). Further studies are needed to assess the clinical implication and the therapeutic relevance of the observation.

In summary, a recurrent fusion of *LMO7-BRAF* is reported in PTC tumors, with a frequency of 2.0% (3/148) in the sample set. Two tumors with the *LMO7-BRAF* fusion are also positive for the *BRAF*^{V600E} mutation. The findings expand the knowledge about the genomic landscape of PTC.

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Author Disclosure Statement

The authors have no conflicts of interest to declare.

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