The Significance of Transcriptomic Signatures in the Multifocal Papillary Thyroid Carcinoma: Two mRNA Expression Patterns with Distinctive Clinical Behavior from The Cancer Genome Atlas (TCGA) Database

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Background and Objectives: The association between multifocal papillary thyroid carcinoma (PTC) and tumor aggressiveness remains controversial. The aim of study is to evaluate molecular subtypes of multifocal PTCs using multiplatform genomic analysis. **Materials and Methods:** Statistical analysis and genomic analysis were performed for gene expression data and clinical data of multifocal PTCs in The Cancer Genome Atlas data. Clinicopathologic findings, recurrence-free survival (RFS), copy number alteration and somatic mutation status in patients in relation to molecular subtypes were analyzed. **Results:** Multiplatform genomic analysis revealed that multifocal PTCs (n=226) were divided into two distinct molecular subgroups. Participants in cluster 2 showed significantly increased risk of extrathyroidal extension, lymph node metastasis, and BRAF^{V600E} mutation compared to patients in cluster 1. To exclude the effect of BRAF mutation and RAS mutation on tumor aggressiveness, we compared clinical parameters between two clusters in patients without BRAF or RAS mutation. Cluster 2 showed significantly higher risk of lymph node metastasis compared to cluster 1. **Conclusion:** Multifocal PTC has two distinct molecular subtypes with distinctive clinical behaviors. Our data suggested the clinical implications of the transcriptomic signature to predict clinical outcomes of multifocal PTC.

Key Words: Thyroid neoplasm, Gene expression profiling, Genomics

Introduction

Papillary thyroid cancer (PTC) is one of the most common endocrine cancers. Its incidence has been rising steadily.¹⁾ PTC presents variously, ranging from a single solitary nodule to multiple co-existing nodules

arising from separate foci in the thyroid gland.^{2,3)} Multifocal pattern in PTC has a high rate ranging from 24% to 46%, among which 50–70% are bilateral.^{4–6)}

The correlation between multifocality and clinical aggressiveness of PTC remains controversial. Many studies reported multifocality is associated with progression of PTC.^{7–9)} However, some of recent studies

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reported opposite result. Singhal et al.¹⁰⁾ reported multifocal PTC showed less extrathyroidal extension (ETE) than solitary nodule. Konturek et al.¹¹⁾ reported that multifocal PTC showed less distant metastasis than solitary nodule. Ahn et al.¹²⁾ reported that odds ratio of lymph node metastasis didn't show significant difference between multifocal and solitary PTC.¹²⁾ In this reason, classification of molecular subtyping of multifocal PTC is absolutely needed to predict clinical behavior of multifocal PTC.

The Cancer Genome Atlas (TCGA) research network group has analyzed multiple genomic platform data of cancers, including PTCs data.¹³⁾ They share valuable data at publicly available websites with diverse clinical information, including mRNA expression using RNA sequence, mutation profile, copy number alteration, micro-RNA expression, methylation profile, and exon sequencing data. Molecular classification of cancer type using these multiplatform data makes it possible to predict clinical behavior and give appropriate treatment, especially for aggressive tumor subtypes.

In this study, using a large set of TCGA mRNA expression data, we classified multifocal PTCs into two subgroups with distinct molecular patterns and different clinical behaviors. Differences in involved pathways between these subgroups were determined using an integrated gene-level approach based on gene expression data, copy number alteration data and somatic mutation data.

Materials and Methods

Genomic and Clinical Data Sets

All genomic data of PTC from TCGA project were obtained from TCGA data portal (<https://tcga-data.nci.nih.gov>) and cancer browser (<https://genome-cancer.ucsc.edu>). Effective samples included both clinical pathological information, including presence of other thyroid disease. Patients with multifocal PTC (n=226) of total 505 participants were enrolled in our study. 80 patients were diagnosed at bilateral PTC, and others represented unilateral PTC. All parameters including age, gender, tumor size, extrathyroidal ex-

tension, TNM stage, presence of thyroid disease, and BRAF mutation state in 226 patients were analyzed. Minimal ETE is defined as tumor cells extending to the sternothyroid muscle or perithyroidal soft tissue, and this is a departure from the 7th edition of the AJCC staging, in which ETE was distributed across T3, T4a, and T4b categories to reflect minimal ETE (including microscopic ETE and macroscopic ETE into strap muscles). Moderate/Advanced ETE, extensive ETE, is defined as tumor of any size that has grown beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, recurrent laryngeal nerve, or major vessels by the tumor. Gene expression data from mRNA-seq, copy number alteration data, somatic mutation data, and clinical parameters in patients with multifocal PTC (n=226) were analyzed. Clinical data included age, gender, tumor size, extrathyroidal extension, TNM stage (AJCC 8th), RAS mutation state, and BRAF mutation state (Supplementary Data 1).

Analysis of Gene Expression Data and Supervised Clustering

All the samples used to test the gene expression are from human thyroid tissue. Effective samples included both clinical and pathological information, and the expression levels of the whole genome (total of 20,531 genes). The expression levels of genes were measured as the reads per kilobase per million reads. Gene expression data were gene-median centered using BRB-ArrayTools software program (<http://linus.nci.nih.gov/BRB-ArrayTools.html>).¹⁴⁾ Gene variability was then computed using median absolute deviation. A total of 3134 most variable genes were selected. A heatmap was generated using Cluster and TreeView software programs.¹⁵⁾

Analysis of Copy Number Alteration Data

Copy number alteration analysis was performed using Cluster and TreeView software programs.

Analysis of Somatic Mutation Data

Somatic mutation analysis was performed using oncoprint at Cbioportal website (<http://cbioportal.org>).

Selection of Specific Gene Signature in Each Group

Genes with a fold change greater than 2 (the absolute value of log 2 fold change greater than 1) and an adjusted p-value less than 0.05 were considered as differentially expressed and were selected genes in groups.

Significant Canonical Signaling Pathways Enriched in Each Subgroup

Pathway analysis was carried using Ingenuity Pathways Analysis (Ingenuity, Redwood City, CA, USA). Genes associated with canonical pathway in Ingenuity Pathways Knowledge Base were analyzed. Significance of association between differentially expressed genes of each group and the canonical pathway was measured using Fischer's exact test at $p < 0.001$.

Statistical Analysis

The association of molecular subtype with each clinical variable was evaluated using Chi-square test. Recurrence free survival (RFS) results between two groups were compared using Kaplan Meier plot and log rank test. Significant difference was considered at $p < 0.05$. All statistical analyses were conducted in R language environment (<http://www.r-project.org>) and SPSS statistics version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Unsupervised Hierarchical Clustering in Multifocal Papillary Thyroid Carcinomas

To find statistically significant gene expression subtypes in multifocal PTCs ($n=226$), hierarchical clustering in an unsupervised and unbiased manner was performed using cluster 3.0. program. Results of hierarchical clustering analysis revealed two molecular subtypes with distinct gene expression profiles (Fig. 1). Red and green colors in the heatmap indicated relative high and low expression, respectively.

Fig. 1. Unsupervised clustering of multifocal papillary thyroid carcinomas (PTCs) revealing two distinct molecular subtypes. A hierarchical clustering of gene expression data from 226 multifocal PTC cases in The Cancer Genome Atlas (TCGA) data. Gene expression levels that were at least 2-fold different in at least 50 cases relative to the median value across cases were selected for hierarchical clustering analysis (3134 gene features). Data are given in matrix format, in which rows represent individual genes and columns represent each patient. Each cell in the matrix represents expression level of a gene feature in an individual pattern. Red and green colors in cell indicate relative high and low expression, respectively, as indicated in the scale bar.

Comparison of Copy Number Alterations Data between Cluster 1 and Cluster 2

As shown in Fig. 2, copy number alterations of cluster 2 were almost none when compared to cluster 1. Cluster 1 multifocal PTCs had some CPN alterations such as chromosome 15,16,17,18,19 amplification and chromosome 20,21 deletion, but cluster 2 multifocal PTCs had chromosome 1q amplification and chromosome 6q deletion.

Comparison of Somatic Mutation Data between Cluster 1 and Cluster 2

Since previous study suggested the importance of

Fig. 2. Copy number (CPN) alteration according to each cluster. The amount of CPN variation of PTC is small (−0.2 to +0.1) when compared to other cancer type. Multifocal PTCs in cluster 1 showed frequent CPN alteration when compared to cluster 2.

both $\text{BRAF}^{\text{V600E}}$ mutation status and RAS score to predict tumor aggressiveness, comparison of somatic mutation data between cluster 1 and cluster 2 were performed (Supplementary Fig. 1). We hypothesized that there was another molecular pathway related to tumor progression in multifocal PTC and the presence of well-known somatic mutation. Interestingly, cluster 2 had more frequent $\text{BRAF}^{\text{V600E}}$ mutation ($n=85/130$, 65.4%) compared to cluster 1. However, $\text{BRAF}^{\text{V600E}}$ mutation was also detected in some patients in cluster 1 ($n=21/96$, 21.9%). Moreover, RAS mutation were detected only cluster 1 (HRAS mutation=7, NRAS mutation=13, and KRAS mutation=2) (Supplementary Fig. 1).

Comparison of Clinicopathologic Parameters between Cluster 1 and Cluster 2

To determine the importance of our molecular sub-

types classification using integrated genomic analysis, we compared clinicopathologic parameters between cluster 1 and cluster 2 using Chi-square test and paired t test (Table 1). There was no significant difference in age, sex, or T stage between cluster 1 and cluster 2. Although tumor size was significantly more increased in cluster 1 compared to that in cluster 2, the status of extra-thyroid extension, lymph node metastasis, and presence of $\text{BRAF}^{\text{V600E}}$ mutation was less frequent in cluster 1 compared to that in cluster 2. In analysis of thyroid disease status between the two groups, nodular hyperplasia was significantly more increased in cluster 1 compared to that in cluster 2. Since several groups identified the relationship of nodular hyperplasia or lymphocytic thyroiditis with tumor aggressiveness,^{16–18)} we investigated the relationship of thyroid disease with clusters. However, there was

Table 1. Case distribution according to cluster 1 and cluster 2

	Cluster		p-value
	Cluster 1 (n=96)	Cluster 2 (n=130)	
Age			0.59
Mean	48.7 (14.3)	47.2 (10.5)	
Gender			0.21
Male	27	48	
Female	69	82	
Size (cm)			0.04
Mean diameter	1.81 (0.9)	1.51 (1.0)	
ETE			<0.0001
None	80	75	
Minimal	16	50	
Moderate/Advanced	0	5	
T stage			0.090
T1	66	102	
T2	26	20	
T3	4	8	
N stage			<0.0001
N0	71	56	
N1a	11	52	
N1b	12	36	
Stage			0.092
Stage I	83	99	
Stage II	11	25	
Stage III	1	6	
Stage IV	1	0	
Unilateral/Bilateral			0.259
Unilateral	67	79	
Bilateral	29	51	
Thyroid disease			0.008
None	51	90	
Lymphocytic thyroiditis	9	17	
Nodular hyperplasia	26	15	
Lymphocytic thyroiditis + nodular hyperplasia	8	8	
BRAF			<0.0001
Wild type	75	45	
V600E mutated	21	85	
RAS			<0.0001
Wild type	76	130	
HRAS mutation	7	0	
NRAS mutation	13	0	

Data are presented as numbers or means (standard deviations).

ETE: extrathyroidal extension, T, N stage: according to AJCC 8th edition

no significant association between lymphocytic thyroiditis and the two molecular subtypes. We also compared RFS between the two clusters. There was no significant difference in RFS between the two groups (Supplementary Fig. 2). Overall, cluster 1 showed more frequent existence of nodular hyperplasia with indolent behavior whereas cluster 2 showed ag-

gressive clinical behavior (Table 1).

The presence of BRAF^{V600E} mutation is one of the most influencing factor in tumor progression. Thus, multivariate analysis using stepwise logistic regression was conducted on parameters shown to be significant in univariate analysis to identify the role of cluster 2 as an independent predictor of the aggressive pheno-

types of multifocal PTC (Table 2). Results of multivariate analysis revealed that cluster 2 was an independent risk factor for lymph node metastasis ($p < 0.0001$, OR: 4.464) and the presence of BRAF^{V600E} mutation ($p < 0.0001$, OR: 5.411). Nodular hyperplasia was also a significantly related factor between two groups ($p = 0.035$, OR: 0.456) (Table 2).

Comparison of Clinicopathologic Parameters between Cluster 1 and Cluster 2 Patients without BRAF Mutation and RAS Mutation

Previous studies have reported that BRAFV600E mutated multifocal PTCs have aggressive phenotype.^{19,20} Consistent with previous results, the pres-

Table 2. Multivariate analysis of clinicopathologic factors between cluster 1 and cluster 2

Factors	Exp(β)	SE	95.0% CI	p-value
Tumor size	0.964	0.570	(0.315, 2.948)	0.948
Minor+moderate/advanced ETE	1.695	0.454	(0.696, 4.131)	0.245
LN metastasis	4.464	0.427	(1.932, 10.313)	<0.0001
Nodular hyperplasia	0.383	0.456	(0.157, 0.935)	0.035
BRAF ^{V600E} mutation	5.411	0.435	(2.307, 12.694)	<0.0001
RAS mutation	0.531	0.144	(0.387, 0.675)	<0.0001

Data analyzed using a stepwise logistic.

CI: confidence interval, ETE: extrathyroidal extension, Exp(β): odds ratio, SE: standard error

Table 3. Case distribution according to cluster 1 and cluster 2 in patients without BRAF or RAS mutation (n=95)

	Cluster		p-value
	Cluster 1 (n=51)	Cluster 2 (n=44)	
Age			0.299
Mean	50.9 (12.1)	47.6 (10.3)	
Gender			0.800
Male	15	14	
Female	36	30	
Size (cm)			0.602
Mean diameter	1.81 (1.2)	1.50 (1.1)	
ETE			0.488
None	40	30	
Minimal	10	12	
Moderate/Advanced	1	2	
T stage			0.370
T1	34	33	
T2	14	7	
T3	3	4	
N stage			<0.001
N0	39	12	
N1a	6	14	
N1b	6	18	
Stage			0.096
Stage I	43	31	
Stage II	8	10	
Stage III	0	3	
Unilateral/Bilateral			0.141
Unilateral	36	24	
Bilateral	15	20	

Data are presented as numbers or means (standard deviations).

ETE: extrathyroidal extension, TNM classification: according to AJCC 8th edition

ence of BRAFV600E mutation was significantly related to our classification using integrated genomic analysis. However, some patients with BRAF^{V600E} mutation included in cluster 1 group showed indolent clinical behavior. This suggests that there is another molecular mechanism independent of mutation of BRAF^{V600E} in multifocal PTCs that is associated with tumor progression. After excluding patients with BRAF mutation (n=114), and RAS mutation (n=21), we re-analyzed clinicopathologic findings between cluster 1 (n=51) and cluster 2 (n=44) (Table 3). Interestingly, presence of lymph node metastasis was significantly associated with cluster 2 group, compared to cluster 1 group (Table 3). These findings support that our classification using integrated genomic analysis may have potential to determine the prognosis of multifocal PTCs, independent of BRAF or RAS mutation status.

Selection of Specific Gene Signature in Each Group

Next, we tried to select genes differentially expressed between cluster 1 and cluster 2 multifocal

PTCs. We applied stringent cutoff criteria ($p < 0.001$, Student t-test; and 2-fold difference) and identified 100 genes for each group (Supplementary Data 2). In Fig. 3, data given in the matrix represent expression level of a gene feature in an individual patient. We selected 100 genes that were cluster specific and drew a heatmap. Red and green colors in the heatmap reflected relative high and low expression, respectively.

Significant Canonical Signaling Pathways Enriched in Cluster 2 Revealed Aggressive Behavior of Multifocal PTC Using Ingenuity Pathway Analysis (IPA)

IPA revealed that each cluster had different canonical signaling pathways (Supplementary Data 3). Diverse immune related pathways such as dendritic cell maturation, Th1 pathway, IL-6 pathway, and Leukocyte Extravasation signaling were enriched in cluster 2. P-value and ratio are presented in Table 4.

Discussion

Using TCGA mRNA expression data, we found that multifocal PTC had two distinct molecular subtypes for the first time. These two subgroups using molecular classification revealed significant different clinical behaviors and different molecular pathways. Multifocal PTC in cluster 1 showed indolent clinical behavior whereas multifocal PTC in cluster 2 showed aggressive clinical behavior, suggesting the importance of integrated genomic analysis for tumor aggressiveness in multifocal PTC. Our study could provide reasons why prognostic effects on tumor progression in multifocality are controversial.

Multifocality is frequently observed in patients with PTC. It is emerging as a prognostic marker in terms of lymph node metastasis and regional recurrence.^{3,6)} However, the prognostic significance of multifocality remains controversial. Moreover, there is no exact biomarker to predict the prognosis of multifocal PTC. Some groups have focused on the importance of bilateral PTC rather than unilateral multifocal PTC in tumor progression of multifocal PTC. However, prognostic effects of bilateral PTC are also controversial.

Fig. 3. Each cluster-specific gene expression patterns shown in heatmap. Data given in the matrix represent the expression level of a gene feature in an individual patient. We selected 100 group-specific genes to draw the heatmap. Red and green colors in cell indicate relative high and low expression, respectively.

Table 4. Altered canonical pathways in cluster 2

Ingenuity canonical pathways	-log (p-value)	Ratio
IL-6 signaling	1.38	0.0469
Dendritic cell maturation	3.9	0.0674
Th1 pathway	4.12	0.0815
Th2 pathway	5.05	0.0867
iCOS-iCOSL signaling in T helper cells	1.96	0.0569
Role of NFAT in regulation of the immune response	0.79	0.0323
Leukocyte extravasation signaling	2.99	0.0569
PKC θ signaling in T lymphocytes	1.42	0.044
NF- κ B signaling	0.544	0.0276
Signaling by Rho family GTPases	0.255	0.0198
Intrinsic prothrombin activation pathway	5.86	0.19
Calcium-induced T lymphocyte apoptosis	1.39	0.0606
B cell receptor signaling	0.283	0.0209
IL-8 signaling	0.263	0.0203

Previous study has suggested that patients with bilateral PTCs show more advance tumor stage, lymph node metastasis, and higher rates of ETE and BRAF^{V600E} mutation than those with unilateral-multifocal PTCs.⁶⁾ Other study has indicated that bilateral and multifocal PTCs have aggressive pathologic features.³⁾ However, only multifocality is significantly associated with an increased risk of disease recurrence.³⁾ Therefore, tumor location is insufficient as a predictive biomarker to treat multifocal PTC. Here, we investigated tumor location of unilateral PTC and bilateral PTC in cluster 1 and cluster 2 using molecular classification (Table 1). We found no significant difference in the proportion of bilateral PTC between the two groups. These results support that molecular classification using integrated genomic analysis may be useful for predicting tumor aggressiveness rather than the location of tumor in multifocal PTC.

Previous studies about papillary microcarcinoma have found that recurrence is significantly higher in the presence of multifocality.²¹⁻²³⁾ However, the effect of multifocality in PTC with tumor size larger than 1 cm is rarely reported. A recent meta-analysis about multifocal PTC has shown that there is no significant association between tumor size and multifocality, although diverse clinic-pathologic parameters including lymph node metastasis, ETE, and disease recurrence are associated with multifocality.²⁾ In this study, we analyzed all multifocal PTC, including microcarcinoma and mac-

rocarcinoma, from TCGA database to evaluate the importance of tumor size in determining tumor progression in multifocal PTCs. Our results are consistent with previous meta-analysis.¹⁾ Tumor size in cluster 2 showing aggressive behavior was significantly smaller than that in cluster 1 showing indolent behavior (Table 1). Additionally, in multivariate analysis (Table 2), there was no significant association between tumor size (>1 cm) and molecular classification, although lymph node metastasis and BRAF^{V600E} mutation were significantly more frequent in cluster 2 compared to those in cluster 1. Thus, tumor size was not a major influencing factor of tumor behavior in multifocal PTC.

Since most studies have focused on the association of clinicopathologic parameters with tumor progression of multifocal PTC without performing molecular analysis, the mechanism involved in the aggressive behavior of multifocal PTC has remains unclear. A recent study has suggested that BRAF^{V600E} mutation, the most well-known mutation related to thyroid cancer prognosis, is significantly associated with tumor progression in multifocal PTC.²⁴⁾ However, several recent studies have failed to corroborate such findings.^{25,26)} Thus, the significance of BRAF^{V600E} mutations in multifocal PTC remains debatable. We analyzed BRAF^{V600E} mutation status between the two clusters to evaluate the effect of BRAF^{V600E} mutation through molecular classifications. Cluster 2 showed significant higher existence of BRAF^{V600E} mutation and more aggressive

clinical behaviors compared to cluster 1. Currently, the presence of BRAF^{V600E} mutation was significantly related to our classification using integrated genomic analysis based on multivariate analysis (Table 2). However, some participants with BRAF^{V600E} mutation were included in cluster 1 with indolent clinical behavior. These results are inconsistent with findings of our previous report.²⁷⁾ When we analyzed TCGA database from all patients with PTC using unsupervised clustering, there were two distinct mRNA expression patterns and all BRAF^{V600E} mutated PTCs were included in one cluster group.²⁷⁾ Molecular mechanisms involved in tumor behavior of multifocal PTCs might be different from those involved in unifocal PTCs. We also investigated clinicopathologic parameters of BRAF^{wild} multifocal PTCs in cluster 1, different from BRAF^{wild} multifocal PTCs in cluster 2, to determine whether our molecular classification using integrated gene-level approach could be used as a surrogate tool to predict prognosis in multifocal PTC independent on BRAF^{V600E} mutation. Interestingly, BRAF^{wild} multifocal PTCs in cluster 1 showed indolent clinical behavior whereas BRAF^{wild} multifocal PTCs in cluster 2 showed aggressive behavior (Table 3), suggesting the prognostic possibility using our molecular classification.

We also analyzed somatic mutation data between the two groups. Multifocal PTC in cluster 1 had frequent RAS family mutations, including NRAS, KRA, and HRAS. In contrast, multifocal PTCs in cluster 2 did not have any RAS mutation. RAS mutation in BRAF^{wild} PTCs is known to be associated with a lower risk of tumor progression.²⁸⁻³⁰⁾ These results are similar to those of our previous study about BRAF^{wild} PTC.²⁷⁾ However, the prevalence of RAS family was detected in only 11% of our data. We also selected genes differentially expressed between cluster 1 and cluster 2 to search for upstream or downstream of functionally important genes. Comparison of differentially expressed genes between the two clusters revealed that there was no different difference in expression of well-known genes such as BRAF, TERT, RAS, RET, or TP53. Interestingly, when we analyzed prominent molecular pathway in cluster 2 compared to that in cluster 1, IPA analysis revealed that many immune-related

signaling including IL-6 signaling and macrophage signaling were related to tumor aggressiveness (Table 4). IL-6 is frequently viewed as a pro-inflammatory cytokine with functions similar to tumor necrosis factor (TNF) and IL-1b in the context of inflammation.³¹⁾ During tumor progression, classically activated macrophages or M1- macrophages are characterized by high expression of IL-6, TNF, and iNOS as well as a dendritic cell-like phenotype to tumor progression, and IL-6 is known to have detrimental effects. Its action is frequently linked to tumor growth, progression, and relapse.³²⁾ Cluster 2 with aggressive behavior in our molecular classification showed upregulated dendritic cell maturation signaling, production of NO and reactive oxygen species in macrophages, Th1 Pathway, and Th2 Pathway, as well as upregulation of IL-6 signaling axis. These findings suggest that IL-6 signaling axis has an important role in thyroid cancer progression. Thus, targeting IL-6 signaling might contribute to clinical management of multifocal PTC patients.

Previous findings have suggested that the pathogenesis of multifocal PTC involves intraglandular spread of a single clone or from independent progenitor clones.³³⁻³⁶⁾ The exact prognostic value of independent clonal origin and intra-thyroidal metastases from a single malignant focus is unknown.³⁶⁾ Currently, it remains inconclusive whether each tumor in multifocal PTC occurs independently or spreads from a single tumor mass in the thyroid gland in our study because TCGA database have gene analysis of single tumor in each patient. To clarify the importance of clonal origin in determining molecular subtypes, a large scaled study using whole genome sequencing of all tumor samples of multifocal PTCs will be necessary. This study identified that multifocal PTC has two distinct molecular subtypes with distinctive clinical behaviors. Using these molecular subtypes, we can predict tumor aggressiveness and make an appropriate treatment plan of multifocal PTC.

In conclusion, multifocal PTC has two distinct molecular subtypes with distinctive clinical behaviors, independently the presence of BRAF or RAS mutation. Our data suggested the clinical implications of the transcriptomic signature to predict clinical outcomes of

multifocal PTC.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Supplementary Fig. 1. Comparison of mutation profiles between cluster 1 and cluster 2.

Supplementary Fig. 2. Comparison of recurrence free survival curve between cluster 1 and cluster 2.