Multi-omics analysis revealed TEK and AXIN2 are potential biomarkers in multifocal papillary thyroid cancer

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Abstract

Background: Papillary thyroid carcinoma (PTC), the most common endocrine cancer, accounts for 80–85% of all malignant thyroid tumors. This study focused on identifying targets that affect the multifocality of PTC. In a previous study, we determined 158 mRNAs related to multifocality in BRAF-mutated PTC using The Cancer Genome Atlas.

Methods: We used multi-omics data (miRNAs and mRNAs) to identify the regulatory mechanisms of the investigated mRNAs. miRNA inhibitors were used to determine the relationship between mRNAs and miRNAs. We analyzed the target protein levels in patient sera using ELISA and immunohistochemical staining of patients’ tissues.

Results: We identified 44 miRNAs that showed a negative correlation with mRNA expression. Using in vitro experiments, we identified four miRNAs that inhibit TEK and/or AXIN2 among the target mRNAs. We also showed that the downregulation of TEK and AXIN2 decreased the proliferation and migration of BRAF (+) PTC cells. To evaluate the diagnostic ability of multifocal PTC, we examined serum TEK or AXIN2 in unifocal and multifocal PTC patients using ELISA, and showed that the serum TEK in multifocal PTC patients was higher than that in the unifocal PTC patients. The immunohistochemical study showed higher TEK and AXIN2 expression in multifocal PTC than unifocal PTC.

Conclusions: Both TEK and AXIN2 play a potential role in the multifocality of PTC, and serum TEK may be a diagnostic marker for multifocal PTC.

Keywords: Papillary thyroid carcinoma, BRAF mutation, Multifocality, Multi-omics

Introduction

Thyroid cancer is the most common malignant endocrine tumor and over the last three decades, its incidence has increased continuously worldwide [1, 2]. The most common form of thyroid cancer, papillary thyroid carcinoma (PTC), accounts for 80–85% of all malignant thyroid tumors [3], and has a favorable prognosis with excellent survival rates. However, a minority of patients with PTC develop locoregional recurrence, including cervical lymph node metastases, which eventually leads to mortality in some patients [4]. PTC often presents with multiple anatomically distinct foci within the thyroid, known as multifocal PTC. The reported prevalence of multifocal PTC ranges from 18 to 87% [5, 6]. However, it remains controversial whether multifocal PTCs are (1) multiple synchronous independent primary tumors or...
The multifocality of PTC has a clinical impact on the continues progress of technology, the importance of microRNAs (miRNAs) have been shown to play pivotal roles in various cancers by regulating the expression of their target mRNAs [17–19]. Recently, studies of miRNAs have focused mostly on malignant neoplasms, and miRNAs have been shown to play pivotal roles in various cancers by regulating the expression of their target mRNAs [17–19].

In this study, we hypothesized that miRNAs interact with overexpressed mRNAs in multifocal BRAF (+) PTCs, regulating protein expression. Therefore, we investigated target miRNAs by exploring the following: (1) screening miRNAs that interact with mRNAs in multifocal BRAF (+) PTCs, (2) validation of miRNAs with functional assays, and (3) protein expression in blood samples from patients with PTCs.

**Materials and methods**

**Data acquisition**

Clinical characteristics and gene expression data (mRNAs and miRNAs) for PTC were downloaded from the Genomic Data Commons Data Portal (https://gdc-portal.nci.nih.gov/). The Cancer Genome Atlas (TCGA) data were available without restrictions on publications or presentations according to TCGA publication guidelines. Patients were categorized according to BRAF mutation status. In addition, patients were divided into two groups according to the multifocality of PTC. Of the 237 patients with BRAF (+) PTCs, 110 had multifocal PTC and 127 had unifocal PTC according to our previous research [9].

**Cell culture**

BCPAP (PTC cell line) harboring the BRAF mutation was purchased from DSMZ Korea and was maintained in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum (FBS) and 100 µg/mL penicillin–streptomycin. All cultures were incubated at 37 °C in the presence of 5% CO₂.

**miRNA (miRNA) inhibitor transfection**

BCPAP cells were transfected with hsa-miR-21a-5p mirVana® miRNA inhibitor (#4,464,084, ID: MH12979, Thermo Fisher Scientific, Waltham, MA, USA), hsa-miR-34a-3p mirVana® miRNA inhibitor (#4,464,084, ID: MH13089, Thermo Fisher Scientific), hsa-miR-203a-3p mirVana® miRNA inhibitor (#4,464,084, ID: MH10152, Thermo Fisher Scientific), hsa-miR-362-3p mirVana® miRNA inhibitor (#4,464,084, ID: MH12485, Thermo Fisher Scientific) and negative control (#4,464,076; Thermo Fisher Scientific). These were used at a final concentration of 30 nM and transfected using Lipofectamine RNAiMAX reagent (Thermo Fisher Scientific).

**miRNA assay**

For assessment of miRNA expression changes, RT-PCR was performed after miRNA inhibitor transfection. Primers for hsa-miR-21a-5p (#A25576, ID: 477,973 mir), hsa-miR-34a-3p (#A25576, ID: 478,047 mir), hsa-miR-203a-3p (#A25576, ID: 478,316 mir), hsa-miR-362-3p (#A25576, ID: 478,058 mir) was purchased from Thermo Fisher Scientific. miRNA was extracted using miNeasy Mini Kit (Qiagen, Germany) and reverse transcription was conducted using a TaqMan Advanced miRNA cDNA Synthesis Kit (Thermo Fisher Scientific). Real time-PCR
was performed using the LightCycler TM system (Roche Applied Science, Indianapolis, IN, USA).

**siRNA transfection**
Negative control (Bioneer, South Korea, #SN-1003) siRNA and target gene siRNA were purchased from Bioneer Corporation (Daejeon, South Korea). Sequences are in Table 1. To create the knockdown cell line, BCPAP cells were seeded at 8 × 10⁴ cells per well in a 6-well plate in RPMI containing 10% FBS. The cells were transfected using DharmaFECT 1 (Thermo Fisher Scientific), as per the manufacturer’s instructions, with 300 nM siRNA and incubated for 48 h.

**Cell proliferation assay**
Cells were seeded at a density of 5 × 10³ cells per well in 96-well plates in RPMI containing 10% FBS. After transfection with siRNA for 72 h, BCPAP cell viability was measured using the Cyto X cell viability assay kit from LPS solution Corporation (South Korea). Cyto X (10 µL) was added to each well and incubated for 1 h in a CO₂ incubator. Optical density (OD) values were quantitatively measured at 450 nm using an enzyme-linked immunosorbent assay reader.

**Real-time PCR**
Total RNA was extracted using the RNeasy mini kit (Qiagen, Germany), miRNA was extracted using miRNeasy mini kit (Qiagen), and cDNA synthesis was performed using a cDNA synthesis kit (Smart gene, South Korea, # SG-CDNAC100). Real-time monitoring of PCR reactions was performed using the LightCycler TM system (Roche Applied Science) and SYBR Green Q-PCR Master Mix with Low Rox (Smart gene, South Korea, #SG-SYBR-ROXL). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control and primer sequences for real-time PCR (Table 2).

**Wound healing assay**
In order to measure the cell migration during wound healing, 48 h after the transfection was carried out (in a 100 mm cell culture dish), BCPAP cells were replated in 24-well plates at a density of 3 × 10⁵ cells per well. Twenty-four hours later, BCPAP cells were cultured in RPMI containing 10% FBS and treated with 1 µg/mL mitomycin-C (Sigma-Aldrich, USA) for 3 h and then wounded with a linear scratch using SPLScar™ Scratcher (SPL Science, South Korea). The average extent of the wound area was evaluated by measuring the width of the wound using ImageJ software.

**Three-dimensional (3D) spheroid formation assay**
3D spheroid formation was examined by culturing the cells in 200 µL complete medium containing 300 cells in each well and cultured in an ultra-low attachment 96-well plate (Corning, USA, #7007). After 1, 3, and 5 days of incubation, spheroid formation was photographed using phase contrast microscopy (4 × magnification).

**Western blot**
Cells were washed with PBS, dissolved in radioimmunoprecipitation assay (RIPA) buffer supplemented, and centrifuged at 15,000 × g for 10 min at 4 °C. Protein concentration was determined by the BCA protein assay (#23,227, Thermo Fisher Scientific) using bovine serum albumin (BSA) as the standard. The proteins were separated by SDS–PAGE and transferred to hybridization nitrocellulose filter membranes (Merck Millipore, USA). The membranes were blocked for non-specific binding with 5% BSA in Tris-buffered saline containing TBS-T (TBS with 0.1% Tween 20) for 1 h at room temperature and then incubated with specific primary antibodies (diluted 1:1000) in TBS-T at 4 °C overnight. After washing with TBS-T three times, the proteins were identified using appropriate secondary antibodies (diluted 1:2000 with 5% BSA). Chemiluminescence was detected with SuperSignal™ West Dura Extended Duration Substrate (Thermo Scientific, USA, #34,075) and visualized using an Amersham Imager 680 (GE Healthcare, USA).

**Blood samples**
Blood samples were collected from 90 patients with BRAF (+) PTCs (42 unifocal PTCs and 48 multifocal PTCs) who underwent total thyroidectomy at Pusan National University Hospital. Samples were collected within one week of surgery. TEK (Mybiosource, USA, #MBS175906), AXIN2 (Mybiosource, USA, #MBS046455), ADAMTS9

### Table 1  Sequence of siRNAs

| siRNA   | Forward(5’–3’) | Reverse(5’–3’) | Target gene |
|---------|----------------|----------------|-------------|
| siTEK   | UGAUGAGGGUGUAGAUCUA | UAGAUCUAACCCCAUCA | TEK         |
| siAXIN2 | GACCAAGGCAUGAUGAA  | UGCCUGAAUGGGUGUGUC | AXIN2       |
| siADAMTS9 | CAGGUAUACACCAACCAACCA | UGGUGGGUGUGUAACCCUG | ADAMTS9     |
| siADAMTL2 | CUCUGUACCCCGGAUGACU | UAGUCAUCGCCGGUACAGA | ADAMTL2     |
levels in serum were evaluated using an ELISA kit according to the manufacturer’s instructions. The results were recorded and analyzed using a microplate reader at 450 nm wavelength (TECAN, Switzerland).

**Immunohistochemistry**

Human tumor tissue paraffin blocks were processed into sections and deparaffinized, followed by incubation with 3% hydrogen peroxide for 20 min to block the endogenous peroxidases. Next, the tissue sections were blocked with 1% bovine serum albumin for 30 min and then incubated with a polyclonal antibody against AXIN2 and TEK (ABclonal Technology; 1:100 dilution) for overnight at 4 °C. Immunoreaction was visualized using the EnVision detection system kit (Dako), and Mayer’s hematoxylin solution was used to stain the nuclei. After staining, images were obtained using an Axio Scan Z1 Digital Slide Scanner (Zeiss, Germany) and analyzed using Zen Blue software (Carl Zeiss. Germany) (magnification: ×100).

**Statistical analysis**

Statistical differences in clinical variables were analyzed using the chi-square test. To determine the relationship between the 145 mRNAs and total miRNAs, we used the Spearman correlation method based on the Hmisc R package (Hmisc version 4.0–3 and R version 3.4.3). From the correlation analysis results, we selected targets with negative correlations of 0.1 or more, and searched the genes related to the selected miRNAs in Tarbase v.8 (http://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=tarbave8) to confirm the correlation. We sorted the selected miRNAs by 13 pathways identified in previous studies. In vitro experimental data were statistical significance of the differences among groups was determined by a one-way analysis of variance (ANOVA) followed by Tukey’s test for multiple comparison using the GraphPad Prism 5 software (GraphPad Software, La Jolla, CA) and differences were considered statistically significant at p < 0.05. A comparison of receiver operating characteristic (ROC) was performed to test the difference between the area under the curve of two ROCs from blood samples using MedCalc 19.8 (MedCalc Software Ltd, Ostend, Belgium).

**Results**

**Screening of potential regulatory miRNAs of 145 mRNAs related to multifocality**

In the previous study, we reported the enriched genes and pathways in multifocal PTC compared to unifocal PTC [9]. Since multifocal PTC is a more aggressive cancer, we focused on the functional role and regulatory mechanisms of the genes based on their pathways.
To obtain more convincing potential regulatory mechanisms, we used multi-omics data from TCGA. 145 mRNAs in the enriched pathways and whole miRNAs were included in the correlation analysis. miRNAs that correlated negatively with mRNAs were discovered in each pathway as follows [9]: 13 miRNAs in axon guidance, 15 in breast cancer, 29 in ectoderm differentiation, 20 in gastric cancer, 5 in the Hippo signaling pathway, 15 in neural crest differentiation, 31 in O-linked glycosylation, 5 in Phospholipase D signaling pathway, 8 in Rap1 signaling pathway, 16 in Wnt signaling pathway, 11 in signaling pathways regulating pluripotency of stem cells, 16 in TCF dependent signaling in response to Wnt, 12 in the Wnt signaling pathy [20–41] (Fig. 1). To select highly relevant genes, the relationship between mRNAs and miRNAs was confirmed by using Tarbase (Table 3 and Additional file 3: Table S1). Combining the results of Tarbase and correlation analysis, we selected four miRNAs (miR21, miR34a, miR203, and miR362) as potential regulators of target mRNAs.

**Selection of target genes through miRNA inhibitors treatment**

To validate the relationship between target mRNAs and miRNAs, BCPAP cells were treated with inhibitors of miR21, miR34a, miR203, and miR362. The expression of each miRNA was suppressed after 24 h (Fig. 2A). Four upregulated genes (TEK, AXIN2, ADAMTS9, and ADAMTSL2) were shown to be miRNA-regulated genes after treatment with miRNA inhibitors (Fig. 2B). Negative results are presented in Additional file 1: Figure S1.

**Functional assays of target mRNAs through siRNA treatment**

The knockdown efficiency was assessed by RT-PCR and western blotting, and the results showed that TEK and AXIN2 expression was knocked down effectively by siRNA in BCPAP cells (Fig. 3A, B and C, and Additional file 2). To study whether this downregulation could inhibit the proliferation of BCPAP cells, their proliferation was monitored. As shown in Fig. 3D, the proliferation rate had significantly decreased on day 3 in
siRNA-transfected cells compared to the negative control. In the 3D spheroid formation assay, the BCPAP cells were transfected prior to the generation of spheroids, which were then allowed to grow for 3 days. After 3 days of treatment with TEK and AXIN2 siRNA, the diameter of 3D spheroids was smaller than that of the negative control group, indicating that the TEK and AXIN2 siRNA complexes could significantly inhibit cell growth (Fig. 3E).

Table 3 The result of correlation analysis. Only values with a correlation coefficient $< -0.1$ and P-value $< 0.05$ were indicated

| Gene symbol | miR203 correlation coefficient | miR203 P-value | miR21 correlation coefficient | miR21 P-value | miR34a correlation coefficient | miR34a P-value | miR362 correlation coefficient | miR362 P-value |
|-------------|-------------------------------|---------------|-------------------------------|---------------|-------------------------------|---------------|-------------------------------|---------------|
| ABCB1       | –                             | –             | –                             | –             | –                             | –             | –                             | –             |
| ADAMTS9     | −0.616                        | 0             | −0.476                        | 6.6E−15       | –                             | –             | –                             | –             |
| ADAMTSL2    | −0.475                        | 7.7E−15       | −0.295                        | 4.0E−06       | –                             | –             | –                             | –             |
| AXIN2       | −0.414                        | 2.6E−11       | −0.271                        | 2.1E−05       | −0.418                        | 1.6E−11       | −0.306                        | 1.00E−06      |
| B4GALT6     | −0.55                         | 3.2E−08       | –                             | –             | –                             | –             | –                             | –             |
| BCA53       | −0.348                        | 8.4E−15       | –                             | –             | –                             | –             | –                             | –             |
| BMPR2       | –                             | –             | –                             | –             | –                             | –             | –                             | –             |
| BOC         | −0.474                        | 3.2E−11       | −0.241                        | 0.000171      | –                             | –             | −0.193                        | 0.0312        |
| CCND2       | –                             | −0.545        | 0                             | −0.418        | 1.6E−11                       | −0.306        | 1.00E−06                      | –             |
| ELOVL2      | −0.371                        | 3.43E−09      | –                             | –             | –                             | –             | –                             | –             |
| ETS1        | −0.435                        | 1.98E−12      | –                             | –             | –                             | –             | –                             | –             |
| HEY2        | −0.435                        | 9.20E−08      | –                             | –             | –                             | –             | –                             | –             |
| HIST1H2AC   | −0.337                        | 0.000301      | –                             | –             | –                             | –             | –                             | –             |
| HIST1H4H    | −0.504                        | 5.93E−14      | –                             | –             | –                             | –             | –                             | –             |
| PIK3R3      | −0.232                        | 6.88E−15      | –                             | –             | –                             | –             | –                             | –             |
| PODXL       | −0.461                        | 6.54E−11      | −0.453                        | 0.76E−13      | –                             | –             | –                             | –             |
| TEK         | −0.406                        | 6.54E−11      | −0.453                        | 0.76E−13      | –                             | –             | –                             | –             |

Fig. 2 Inhibition of miR21, miR34a, miR203, and miR362 in BCPAP cells. A miRNA inhibitor transfection reduced the expression levels of all four miRNAs by more than 40% in BCPAP as analyzed by qPCR. B AXIN2, ADAMTS9 and ADAMTSL2 mRNA expression was up-regulated by miR21 inhibitor (gray), TEK, AXIN2 mRNA expression was up-regulated by miR34a inhibitor (green). And AXIN2, ADAMTS9 and ADAMTSL2 mRNA expression was up-regulated by miR203 inhibitor (orange), AXIN2 mRNA expression was up-regulated by miR362 inhibitor (blue). *P < 0.05, **P < 0.01, ***P < 0.001, compared to the control.
cells, we performed a cell migration assay. In a wound-healing assay, the artificial wound gap in plates of the negative siRNA-transfected BCPAP cells was significantly narrower than that of the $\text{TEK}$- and $\text{AXIN2}$-siRNA-transfected BCPAP cells at 6 h (Fig. 3F). $\text{ADAMTS9}$ and $\text{ADAMTSL2}$ were excluded from the experiment because of the repeated heterogeneous results.

**TEK and AXIN2 from patients with BRAF (+) PTCs: multifocal vs unifocal**

42 patients with BRAF (+) unifocal PTCs and 48 patients with BRAF (+) multifocal PTC were included in this analysis. The sizes of the largest tumors were not significantly different ($P = 0.5710$) between unifocal (1.6 ± 0.7 cm) and multifocal (1.7 ± 0.8 cm) PTCs. The level of TEK in serum from multifocal PTCs was higher than that from unifocal PTCs ($P < 0.0001$, Fig. 4A), while the level of AXIN2 from multifocal PTCs was lower than that from unifocal PTCs ($P < 0.0001$, Fig. 4B). To test the performance of TEK and AXIN2, the areas under the curves were compared, which were not significantly different (TEK 0.854; AXIN2 0.779) (Fig. 4C). In the IHC results, the TEK and AXIN2 expression in multifocal PTC are much higher than unifocal PTC (Fig. 4D).

**Discussion**

The results of the current study are summarized as follows: (1) 13 miRNAs interacted with mRNAs overexpressed in multifocal BRAF (+) PTCs, (2) after validation with miRNA inhibitors, and functional assays, the mRNA expression of $\text{TEK}$ and $\text{AXIN2}$ was associated with the multifocality of BRAF (+) PTCs, (3) TEK and AXIN2 in blood samples of patients with multifocal PTCs were significantly different from those with unifocal PTCs.

PTC is the most common form of thyroid cancer with a favorable prognosis; however, a minority of patients develop locoregional recurrence, which eventually leads to mortality in some of these patients [4]. Risk factors that affect the risk of recurrence in PTC include extrathyroidal extension, lymph node involvement, BRAF mutation status, tumor size, and sex [42]. Multifocal PTCs, defined as the presence of two or more than 2 anatomically separated tumor foci in the thyroid gland [43], have been associated with an increased risk of lymph node and distant metastases, as well as disease recurrence [44]. In clinical settings, physicians often encounter patients with multiple thyroid nodules; however, fine needle aspiration of multiple nodules is rarely performed. The selection of thyroid nodules for fine needle aspiration is mainly determined by the cancer probability from ultrasonographic...
findings, such as the content of the nodule, echogenicity, shape, margin, calcification, and vascularity [45]. According to the ATA guidelines [4], hemithyroidectomy is sufficient for unifocal PTCs without prior irradiation of the head and neck area, a history of familial thyroid cancer, and known lymph node metastasis. In contrast, total thyroidec
tomy can be recommended if the nodules are confirmed to be malignant in the bilateral lobes [46]. Therefore, decisions regarding the optimal extent of surgery for patients with multiple thyroid nodules should be made after careful consideration.

Based on the results of the correlation analysis of the TCGA data and the in vitro experiments, we identified the oncogenic role of TEK and AXIN2 through miRNA regulatory mechanisms. Although we did not find the exact role of miRNAs in PTC, we observed that 4 miRNAs (miR21, miR34a, miR203, and miR363) inhibition increased the expression of AXIN2 while TEK is only affected by miR34a in BRAF (+) PTC cells. Along with the discovery of the candidate regulatory mechanisms, four miRNAs and two mRNAs were also validated in this study.

TEK (i.e., Tie2) is a receptor tyrosine kinase, which is mainly expressed in endothelial cells and controls vascular regeneration and stabilization [47]. The angiopoietin-Tie system is known to be involved in inflammation, metastasis, and lymphangiogenesis; therefore, multiple clinical trials were performed with selective Tie2 inhibitors [48]. However, the role of TEK in cancer cells remains unclear. Knockdown of TEK increased the proliferation and migration of clear cell renal cell carcinoma [49]; however, the overexpression of TEK in glioma cells was associated with tumor malignancy and drug resistance [50, 51]. Therefore, TEK in BRAF (+) PTC cells may affect cell proliferation, invasion, and multifocality.

Since AXIN2 acts as an inhibitor of canonical Wnt signals in normal cells, many studies have focused on elucidating their role as tumor suppressors. However, silencing AXIN2 decreases the invasive and metastatic characteristics of colon cancer [52]. In the current study, AXIN2 expression was higher in multifocal PTC than in unifocal PTC, and knockdown of AXIN2 inhibited cell proliferation in BRAF (+) PTC cells. In addition, it was found that the expression of Wnt target
genes, including AXIN2, differed based on the presence/absence of the BRAF mutation [53]. Our previous results showed that AXIN2 expression did not differ between BRAF (−) multifocal and unifocal PTC [9]. Taken together, these results suggest that the role of AXIN2 may be different for each cancer, and BRAF mutations may affect AXIN2 expression.

In this study, the level of TEK in the serum from multifocal PTC patients was higher than that in unifocal PTCs, while the level of AXIN2 from multifocal PTCs was lower than that from unifocal PTCs. On the contrary, a lower level of AXIN2 was observed in multifocal PTC. The biological reason for this phenomenon may be the half-life of the protein [54, 55] as well as post-transcriptional and post-translational modifications. According to a recent analysis, protein expression correlates with the corresponding mRNA level by 20–40%, and mRNA expression levels are not completely representative of the corresponding protein concentration [56, 57]. Therefore, serum TEK and AXIN2 levels may provide information on multifocality in patients with BRAF (+) PTC in a separate way.

Summarily, we used big database to obtain potential regulatory mechanism of target genes and highlighted the interaction of miRNAs with the expression of target genes and proteins in multifocal BRAF (+) PTC. miRNA inhibition increased the mRNA expression of TEK and AXIN2. Serum TEK and AXIN2 levels may provide information on multifocality of BRAF (+) PTC. Although there are some limitations, this study provides evidences for the regulatory mechanism of the genes and their contribution to PTC multifocality for the first time.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12935-022-02606-x.

Additional file 1: Figure S1. Expression changes of mRNAs in BRAF (+) BCPAP cells after treatment with miR21 (gray), miR34a (green), miR203 (orange), and miR362 (blue) inhibitors at 24hr.

Additional file 2: Figure S2. Raw Western blot data.

Additional file 3: Table S1. The results of Tarbase.

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Author contributions
GH, HJ, JW, YH, and KJ wrote the article. GH, HJ, EK, and SE performed experimental evaluation. JW, KY, and U analyzed omics data. SH and BJ designed figures. YH, and KJ supervised the article. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The biospecimens and data used for this study were provided by the Biobank of Pusan National University Hospital, a member of the Korea Biobank Network. Informed consent for the collection, storage, and use of blood samples was obtained from each patient. This study was approved by the Institutional Review Board of Pusan National University Hospital (PNUH-2005-013-091).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Lee JH, Lee ES, Kim YS. Clinicopathologic significance of BRAF V600E mutation in papillary carcinomas of the thyroid: a meta-analysis. Cancer. 2007;110(1):38–46.
2. Ciampi R, Mian C, Fugazzola L, Cosci B, Romei C, Barollo S, et al. Evidence of a low prevalence of RAS mutations in a large medullary thyroid cancer series. Thyroid. 2013;23(1):50–7.
3. Cabanillas ME, McFadden DG, Durante C. Thyroid cancer. Lancet. 2016;388(10061):2783–95.
4. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016;26(1):1–133.
5. Wang W, Su X, He K, Wang Y, Wang H, Wang H, et al. Comparison of the clinicopathologic features and prognosis of bilateral versus unilateral multifocal papillary thyroid cancer: an updated study with more than 2000 consecutive patients. Cancer. 2016;122(2):198–206.
6. Iacobone M, Jansson S, Barczynski M, Gorzetzi P. Multifocal papillary thyroid carcinoma—a consensus report of the European Society of Endocrine Surgeons (ESES). Langenbecks Arch Surg. 2014;399(2):141–54.
7. Iida F, Yonekura M, Miyakawa M. Study of intraglandular dissemination of thyroid cancer. Cancer. 1969;24(4):764–71.
8. Russell WD, Ibanez ML, Clark RL, White EC. Thyroid carcinoma: Classification, intraglandular dissemination, and clinicopathological study based upon whole organ sections of 80 glands. Cancer. 1963;16:1425–60.
9. Pak K, Suh S, Goh TS, Kim SJ, Oh SO, Seek JW, et al. BRAF-positive multifocal and unifocal papillary thyroid cancer show different messenger RNA expressions. Clin Endocrinol. 2019;90(4):601–7.
10. Shattuck TM, Westra WH, Ladenson PW, Arnold A. Independent clonal origins of distinct tumor foci in multifocal papillary thyroid carcinoma. N Engl J Med. 2005;352(23):2406–12.

10. Shattuck TM, Westra WH, Ladenson PW, Arnold A. Independent clonal origins of distinct tumor foci in multifocal papillary thyroid carcinoma. N Engl J Med. 2005;352(23):2406–12.
11. Kim HJ, Sohn SY, Jang HW, Kim SW, Chung JH. Multifocality, but not bilaterality, is a predictor of disease recurrence/persistence of papillary thyroid carcinoma. World J Surg. 2013;37(2):376–84.

12. Grant CS, Hay JD, Gough IR, Bergstralh EJ, Goellner JR, McConahey WM. Local recurrence in papillary thyroid carcinoma: is extent of surgical resection important? Surgery. 1988;104(6):954–62.

13. Bilimoria KY, Bentrem DJ, Ko CY, Stewart AK, Winchester DP, Talamentis MS, et al. Extent of surgery affects survival for papillary thyroid cancer. Ann Surg. 2007;246(3):375–81 [discussion B14].

14. Cai Y, Yu X, Hu S, Su J. A brief review on the mechanisms of miRNA regulation. Genom Proteom Bioinform. 2009;7(4):147–54.

15. Kim K, Ko Y, Oh H, Ha M, Kang J, Kwon EJ, et al. MicroRNA-98 is a prognostic factor for asbestosis-induced mesothelioma. J Toxicol Environ Health A. 2020;83(3):126–34.

16. Drakaki A, Iliopoulos D. MicroRNA gene networks in oncogenesis. Curr Genom. 2009;10(10):35–41.

17. Choudhury Y, Tay FC, Lam DH, Sandaranjar E, Tang C, Ang BT, et al. Attenuated adenosine-to-inosine editing of microRNA-3’76a promotes invasiveness of glioblastoma cells. J Clin Invest. 2012;122(11):4059–76.

18. Hayes J, Peruzzi PP, Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. Trends Mol Med. 2014;20(8):460–9.

19. Stahlhut C, Slack FJ. MicroRNAs and the cancer phenotype: profiling, signatures and clinical implications. Genome Med. 2013;5(12):111.

20. Bilalshahal T, Balasubramanian K, Brown J, Ramkrishnan A, Tokor-Orji S, Kabos P, et al. Genome-wide analysis of miRNA-mRNA interactions in marrow stromal stem cells. 2014;32(3):662–73.

21. Boudreau RL, Jiang P, Gilmore BL, Spengler RM, Tirabassi R, Nelson MS, et al. Extent of surgery affects survival for papillary thyroid cancer. Surgery. 1988;104(6):954–62.

22. Brock M, Trenkmann M, Gay RE, Michel BA, Gay S, Fischler M, et al. MicroRNA-210 is upregulated in malignant thyroid epithelial cells and is a predictor of disease recurrence/persistence of papillary thyroid carcinoma. J Lipid Res. 2015;56(1):38–50.

23. Cantini L, Isella C, Pettic C, Picco G, Chiola S, Ficarra E, et al. MicroRNA-mRNA interactions underlying colorectal cancer molecular subtypes. Nat Commun. 2015;6:8878.

24. Cao J, Shen Y, Zhu L, Xu Y, Zhou Y, Wu Z, et al. miR-129-3p controls cilia assembly by regulating CP110 and actin dynamics. Nat Cell Biol. 2012;14(7):697–706.

25. Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee JD, et al. Transcription-wide discovery of miRNA-mRNA interactions in mouse liver. Mol Cell. 2007;26(5):745–52.

26. Gabriel G, Wurdinger T, Kesari S, Esau CC, Burchard J, Linsley PS, et al. MicroRNA-21 promotes glioma invasion by targeting matrix metalloproteinase regulators. Mol Cell Biol. 2008;28(17):S369–80.

27. Gottwein E, Corcoran DL, Mukherjee N, Skalsky RL, Hafner M, Kaller M, et al. Genome-wide analysis of miRNA-mRNA interactions in marrow stromal stem cells. 2014;32(3):662–73.

28. Haecker I, Gay LA, Yang Y, Hu J, Morse AM, McIntyre LM, et al. Ago HITS-CLIP expands understanding of Kaposi’s sarcoma-associated herpesvirus miRNA function in primary effusion lymphoma cell lines. PLoS Pathog. 2012;8(1):e1002484.

29. Wang K, Wang X, Zhou J, Zhang A, Wan Y, Pu P, et al. miR-92b controls glioma proliferation and invasion through regulating Wnt/beta-catenin signaling via Nemo-like kinase. Neuro Oncol. 2013;15(5):578–88.

30. Xue Y, OuYang K, Huang J, Zou Y, OuYang H, Li L, et al. Direct conversion of fibroblasts to neurons by reprogramming PTB-regulated microRNA circuits. Cell. 2013;152(2–3):82–96.

31. Zahedi A, Bondaz D, Rajaraman M, Leslie WD, Jefford C, Young JE, et al. Risk for thyroid cancer recurrence is higher in men than in women independent of disease stage at presentation. Thyroid. 2020;30(6):871–7.

32. Genpeng L, Jianyong L, Jiaying Y, Ke J, Zhihui L, Rixiang G, et al. Independent predictors and lymph node metastasis characteristics of multifocal papillary thyroid carcinoma. Medicine. 2018;97(5):e9619.

33. Joseph KR, Edrissmanne S, Edsick GL. Multicentricity as a prognostic factor in thyroid cancer: a meta-analysis. Int J Surg. 2018;50:121–5.

34. Shin JH, Baek JH, Chung J, Ha EJ, Kim JH, Lee YH, et al. Ultrasongraphy diagnosis and imaging-based management of thyroid nodules: revised Korean society of thyroid radiology consensus statement and recommendations. Korean J Radiol. 2016;17(3):170–95.

35. Xue S, Wang P, Liu J, Chen G. Total thyroidectomy may be more reasonable as initial surgery in unilateral multifocal papillary thyroid microcarci­noma: a single-center experience. World J Surg Oncol. 2017;15(1):62.

36. Ha M, Son YR, Kim J, Park SW, Hong CM, Choi D, et al. TEK is a novel prognostic marker for clear cell renal cell carcinoma. Eur Rev Med Pharmacol Sci. 2019;23(4):1451–8.

37. Huang H, Bhat A, Woodnutt G, Lappe R. Targeting the ANGPT-TIE2 pathway in malignancy. Nat Rev Cancer. 2010;10(8):575–85.

38. Chen S, Yu M, Ju L, Wang G, Qian K, Xiao Y, et al. The immune-related biomarker TEK inhibits the development of clear cell renal cell carcinoma (cRCC) by regulating AKT phosphorylation. Cancer Cell Int. 2012;12:119.

39. Martin V, Xu J, Pabissetty SK, Alonso MM, Liu D, Lee OH, et al. Tied2-mediated multidrug resistance in malignant gliomas is associated with upregulation of ABC transporters. Oncogene. 2009;28(24):2358–63.

40. Lee OH, Xu J, Fueyo J, Fuller GN, Aldape KD, Alonso MM, et al. Expression of the receptor tyrosine kinase Tie2 in neoplastic glial cells is associated with integrin beta1-dependent adhesion to the extracellular matrix. Mol Cancer Res. 2006;4(12):915–26.

41. Wu QZ, Li YY, Hu CY, Ford M, Kleer CG, Weiss SJ. Canonical Wnt signaling regulates IL-1beta induced miRs-21-3p and -27a-5p in human aortic endothelial cells. J Lipid Res. 2015;56(1):38–50.

42. Zahedi A, Bondaz D, Rajaraman M, Leslie WD, Jefford C, Young JE, et al. Risk for thyroid cancer recurrence is higher in men than in women independent of disease stage at presentation. Thyroid. 2020;30(6):871–7.

43. Genpeng L, Jianyong L, Jiaying Y, Ke J, Zhihui L, Rixiang G, et al. Independent predictors and lymph node metastasis characteristics of multifocal papillary thyroid carcinoma. Medicine. 2018;97(5):e9619.

44. Joseph KR, Edrissmanne S, Edsick GL. Multicentricity as a prognostic factor in thyroid cancer: a meta-analysis. Int J Surg. 2018;50:121–5.

45. Shin JH, Baek JH, Chung J, Ha EJ, Kim JH, Lee YH, et al. Ultrasongraphy diagnosis and imaging-based management of thyroid nodules: revised Korean society of thyroid radiology consensus statement and recommendations. Korean J Radiol. 2016;17(3):170–95.
56. Tian Q, Stepaniants SB, Mao M, Weng L, Feetham MC, Doyle MJ, et al. Integrated genomic and proteomic analyses of gene expression in mammalian cells. Mol Cell Proteom. 2004;3(10):960–9.

57. Nie L, Wu G, Zhang W. Correlation between mRNA and protein abundance in Desulfovibrio vulgaris: a multiple regression to identify sources of variations. Biochem Biophys Res Commun. 2006;339(2):603–10.

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