의학박사 학위논문

Association Between Somatic Mutation and Clinical Features in Korean Patients with Thyroid Cancer

한국인 갑상선암의 체성돌연변이의 빈도와 임상상과의 연관성 연구

2015 년 8 월

서울대학교 대학원

의학과 분자유전체의학 전공

임 정 아

한국인 갑상선암의 체성돌연변이의 빈도와 임상상과의 연관성 연구

지도교수 박 영 주

이 논문을 의학박사 학위논문으로 제출함

2015 년 4 월

서울대학교 대학원

의학과 분자유전체의학 전공

임 정 아

임정아의 의학박사 학위논문을 인준함

2015 년 6 월

위 원 장 (인)

부위원장 (인)

위 원 (인)

위 원 (인)

위 원 (인)

Association Between Somatic Mutation and Clinical Features in Korean Patients with Thyroid Cancer

by
Jung Ah Lim

A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Medicine (Molecular and Genomics) at Seoul National University College of Medicine

July 2015

Approved by Thesis Committee:

Professor _____ Chairman
Professor _____Vice chairman
Professor _____
Professor _____
Professor _____

Abstract

Association Between Somatic Mutation and Clinical Features in Korean Patients with Thyroid Cancer

Jung Ah Lim

Major in Molecular and Genomics

Department of Medicine

Seoul National University Graduate School

Introduction : Thyroid cancer incidence rates have increased steadily over the last 40 years, this it is the most common malignancy worldwide. Although main cause of increase is generally thought as the early diagnosis of thyroid cancer using ultrasonography, some environmental and genetic factors also have been suggested. In Korea, the reported incidence of BRAF mutation in papillary thyroid cancer (PTC) is around 70-80% showing the highest rate all

over the world. On the other hand, the frequency of other genetic mutation such as RAS mutation, RET/PTC or PAX8/PPARγ rearrangements was considered to be relatively low. The purpose of this study was to elucidate the prevalence of somatic mutation in this BRAF-V600E-prevalent area and its clinical implication

Materials and Methods : We reviewed 606 BRAF-wild type PTC and 174 follicular thyroid cancer (FTC) cases who underwent total thyroidectomy at Seoul National University Hospital between 1997 and 2012. RAS and TERT point mutations of tumor DNA or RET, ALK and PPARγ rearrangements in the postoperative formalin-fixed paraffin-embedded samples were assessed by using direct sequencing, immunohistochemistry or fluorescence in situ hybridization (FISH).

Results : RAS point mutations were shown in 9.2% of BRAF-wild type PTCs, consisting of 18 N-RAS 61, 1 RAS 60, 2 K-RAS 12/13 and 1 H-RAS 61 mutations, and an estimated rate of RAS mutation in all PTCs was 2.4%. The frequency of RET rearrangement was 8.8% of BRAF-WT PTC, and its estimated frequency among all PTC was 2.6%. RAS mutation was

significantly more common in follicular variant PTC than in conventional type (29.2% vs 2.8%, p<0.001). Among all type of PTC, the prevalence of conventional type was 80.1% and that of follicular variant type was only 14.8%. Mean tumor size is significantly larger (1.9 ± 1.5cm vs 1.2 ± 0.8cm, *p*=0.027), but LN metastases and are less common in RAS mutant PTC, compared with RAS-wild type PTC (4.5% vs 40.2%, *p*=0.001). ALK rearrangements were found in 4 cases of 218 BRAF-wild type PTCs (1.8%) by using immunohistochemistry and FISH analysis, and an estimated rate of ALK rearrangements in all PTC was 0.5%. TERT mutations were found in 18 of 433 PTC cases (4.2%). Tumor recurrence was 41 of 373 (11.0%) in TERT mutations-negative cases versus 6 of 18 (33.3%) in TERT mutation-positive cases (hazard ratio [HR] 3.38; 95% CI 1.87 to 8.42; *p* = 0.005). Disease-free survival curves displayed a moderate decline with TERT mutation. In FTC, RAS mutation were found in 44 (23.4%) among 148 patients, but there were no significant difference between RAS-mutant and RAS-wild type FTC groups. Two PPARγ rearrangements (3.3%) were discovered in 61 FTC, but we found no PPARγ rearrangement in follicular variant PTC. TERT mutations

were found in 7 of 120 FTC cases (5.8%). Tumor recurrence was 6 of 109 (5.5%) in TERT mutations-negative cases versus 2 of 7 (28.6%) in TERT mutation-positive cases (HR 7.22; 95% CI 1.39 to 37.45; $p = 0.019$).

Conclusions : The prevalence of RAS mutation in Korea was relatively low, due primarily to low proportion of follicular variant in all PTCs. The percentage of RET rearrangements also showed low, suggesting different etiologic factors in Korea, compared with other countries. TERT promoter mutations were associated with aggressive clinicopathological outcomes in both PTC and FTC.

Keywords: papillary thyroid cancer, follicular thyroid cancer, somatic mutation, gene rearrangement

Student number: 2012-30560

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PTC: papillary thyroid carcinoma

FTC: follicular thyroid carcinoma

MAPK: mitogen activated protein kinase

TERT: telomerase reverse transcriptase

ALK: anaplastic lymphoma kinase

WBS: whole body scan

RAI: radioactive iodine

IHC: immunohistochemistry

FFPE: formalin fixed paraffin-embedded

FISH: fluorescence in situ hybridization

ETE: extrathyroidal extension

LN: lymph node

HR: hazard ratio

TCGA: The Cancer Genome Atlas

Introduction

Thyroid cancer is the most common endocrine malignancy in Korea, with a rapidly rising incidence worldwide in recent years (1, 2). Although the main reason of increase in diagnosing the thyroid cancer is generally thought as the screening of thyroid nodule by ultrasonography, some environmental and genetic factors cannot be neglected with some evidences including the genetic mutation of thyroid cancer (1). Since exposure to ionizing radiation during childhood is a well-established risk factor for thyroid cancer (3, 4), it is conceivable that the rising incidence may reflect increasing use of medical radiation procedures, such as diagnostic computed tomography scans (5, 6). Other possible temporally relevant risk factors include body mass index, smoking, environmental chemicals, and reproductive patterns (7).

Previous studies have suggested and established several driver mutations in thyroid carcinoma, and three of them, which activate mitogen activated protein kinase (MAPK) signaling pathway, account most of thyroid cancer subjects; rearrangements of RET and NTRK1 tyrosine kinases and activating

mutations of BRAF and RAS (8, 9). Among them, RAS is a classical dual activator of MAPK and the phosphatidylinositol 3-kinase/AKT pathways. The RAS genes including NRAS, HRAS, and KRAS are known as protooncogenes. Several solid tumors such as thyroid, pancreas, biliary, and colon cancers and melanoma carry RAS mutations. It is reported that the frequency of RAS mutations in thyroid cancer is about 10-20%, and many thyroid cancers carry mutations in all three isoforms (10). Activating point mutations generally affect codons 12,13, and 61 of the RAS genes. Among them, the most common mutations are NRAS codon 61 and HRAS codon 61 in thyroid cancer. RAS mutations are detected in all kinds of thyroid cancers with various frequencies, including 10-20% of papillary carcinomas, 40-50% of follicular carcinomas and 20-40% of poorly differentiated and anaplastic carcinomas (11-13).

Among papillary thyroid carcinomas (PTC), virtually all tumors that harbor a RAS mutation grow forming neoplastic follicles and no papillary structures and are, therefore, diagnosed as the follicular variant of papillary carcinoma (14, 15). However, also in benign thyroid follicular adenomas, RAS mutations

are found (16-18). This mutational finding in benign adenomas as well as in follicular-patterned carcinomas suggest that RAS-positive follicular adenomas may serve as a precursor for RAS-positive follicular carcinomas and the follicular variant of papillary carcinomas (9). Moreover, RAS mutations may predispose well-differentiated cancers to dedifferentiation and anaplastic transformation (19, 20).

RET/PTC is a chromosomal rearrangement found in PTC (21). As a result of the rearrangement, a portion of RET gene is fused to one of several counterpart genes. All chimeric genes contain the portion of RET that encodes the intact tyrosine kinase domain of the RET protein fused to an active promoter of another gene that drives the expression and ligand-independent dimerization of the RET/PTC protein, which leads to chronic stimulation of MAPK signaling and tumorigenesis in thyroid cells (22-24). The two most common rearrangement types are RET/PTC1 and RET/PTC3, in which RET is fused to either CCDC6 (also known as H4) or NCOA4 (also known as ELE1 or RFG), respectively (25, 26). Both of these rearrangement types are paracentric, intrachromosomal inversions, as all fusion partners reside on the

long arm of chromosome 10 (27, 28). In contrast, RET/PTC2 and nine more recently discovered types of RET/PTC rearrangements are all interchromosomal rearrangements formed by RET fusion to genes located on different chromosomes (29-34).

A strong association exists between RET/PTC rearrangements and exposure to ionizing radiation, which is a well-known risk factor for PTC. RET/PTC rearrangements are detected in up to 80% of PTC in individuals exposed to either accidental radiation (mostly radioiodine) or therapeutic radiation (mostly external beam) (35-38). The frequencies of RET/PTC3 rearrangement type and novel types of RET/PTC were increased due to radiation exposure after Chernobyl accident (35-38). In contrast, the point mutations including the BRAF and RAS genes are rare in radiation-related tumors but common in the general population (39). Among PTC of atomic bomb survivors in Japan (where doses received by the thyroid gland were calculated with high precision), an increased frequency of RET/PTC rearrangement showed a strong positive correlation with increased radiation dose, whereas frequency of BRAF point mutations showed an inverse correlation with the radiation

dose (40, 41).

Recently, rearrangements of anaplastic lymphoma kinase (ALK) that lead to both ectopic expression and constitutive activation of the ALK fusion protein were reported in differentiated thyroid carcinoma and anaplastic carcinoma (42, 43). Treatment with clinically available ALK inhibitors, such as crizotinib and TAE 684, yielded in vitro antitumor efficacy (43) and produced clinical improvement in anecdotal cases (42, 44). To introduce these ALK inhibitors for the clinical treatment of thyroid cancer, the precise frequency of the ALK rearrangement in a wide range of thyroid tumors should be evaluated first. Since the expression of ALK in non-small cell lung cancer is extremely limited during adulthood and occurs only in cancer tissues following chromosomal rearrangement, immunohistochemistry (IHC) may be a useful tool for screening for rearrangements of ALK in thyroid cancer. Thus, in thyroid cancer a practical diagnostic assay for identifying rearrangements of ALK is essential.

PAX8/PPARγ rearrangement leads to the fusion between a portion of the PAX8 gene, which encodes a paired domain transcription factor, and the

PPARγ gene (45). The fusion results in strong overexpression of the chimeric PAX8/PPARγ protein (45, 46), although the mechanisms of its transforming activity remain to be fully understood. PAX8/PPARγ is a prototypic alteration found in follicular thyroid carcinoma (FTC), where it occurs with a frequency of 30-35% (47, 48). In several studies, this rearrangement has also been found in some (2-13%) follicular adenomas and in a low proportion (1-5%) of the follicular variant of PTC (47-49), although occasional reports of a much higher prevalence of this rearrangement in the follicular variant of PTC also exist (50).

Recently highly frequent mutations in the promoter region of telomerase reverse transcriptase (TERT) were identified in various human malignancy including thyroid cancer (51, 52). TERT mutations occur in two hot spot positions, chr5: 1,295,228C>T and chr5:1,295,250C>T (termed here as C228T and C250T respectively) (52). These mutations confer TERT increased transctriptional activities by creating binding sites for ETS (E twenty-six) transcription factors in the TERT promoter, providing a mechanism for the overexpression of TERT observed in human cancers (53, 54). Several reports

showed that TERT promoter mutations are associated with aggressive features and with the presence of BRAF or RAS mutations in differentiated thyroid cancer as well as poorly differentiated cancer (55-58) .

There were two different reports that mutational profiles of thyroid cancer changed over time in the United States and Italy (59, 60). One showed that the percentage of RAS mutations rose sharply after 2000, whereas the overall proportion of BRAF mutations remained stable (59). By contrast, there were a significant decrease in the prevalence of RET/PTC rearrangements and an increase in BRAF V600E mutations in another study (60). However, it is known that the prevalence of BRAF V600E in Korean PTC patients is higher than in the average rate of other countries (61, 62). There are a few report that RAS mutation or RET/PTC rearrangement of thyroid cancer in Korea is relatively low (63, 64). Thus an evaluation of the genetic mutation in Korean thyroid cancer patients and how changes in these molecular profiles have influenced the clinicopathologic features of thyroid cancer over time is important. In addition, since the frequency and clinical impact of TERT promoter mutation in Korean thyroid cancer were not evaluated well yet, we

conducted mutational analysis for TERT mutation in differentiated thyroid cancer. In this study, we investigated the mutational profile of Korean thyroid cancers and its changing trend over 10 years and evaluated its clinical implication.

Materials and Methods

Patients and Methods

This study included patients who underwent a thyroidectomy and were diagnosed as histologically PTC (606 patients) and FTC (174 patients) in Seoul National University Hospital. Of these patients, 404 underwent surgery between April 1995 and June 2003 and 376 underwent surgery between January 2009 and July 2012. Patients with follicular adenoma (n=44) were also evaluated to compare with FTC. Medical charts of patients were reviewed retrospectively, and the TNM classification system was used for pathologic staging. All histologic slides were reviewed to verify and standardize the histologic diagnosis using the current World Health Organization criteria. The BRAF V600E mutation was prescreened in all cancer samples using direct sequencing. The study protocol was approved by the institutional review boards of Seoul National University Hospital.

All patients were treated with one of three types of surgical resection: total thyroidectomy, subtotal thyroidectomy, or lobectomy. Therapeutic neck

dissections were conducted for standard indications, and empirical central or anterior lymph node dissections have been performed since 2003. Postoperative treatment included conventional thyroid stimulating hormone suppression and radioactive iodine (^{131}I) ablation. The ablation was conducted in patients with lymph node metastases, extrathyroidal extension, or distant metastasis until no further iodine uptake was detected on a postablation whole body scan (WBS). The dose of radioactive iodine (RAI) depended on the individual pathological characteristics of the PTC. In patients with favorable pathological characteristics, 30 mCi of RAI was administered whereas a higher dose of RAI (100 to 200 mCi) was used for patients with aggressive pathological characteristics.

Recurrent and persistent diseases were determined by the results of subsequent surgery, serum thyroglobulin level, neck ultrasound or computed tomography, ^{131}I WBS, bone scan, or F-fluorodeoxyglucose positron emission tomography scan.

DNA isolation

The tumor areas were marked by an endocrine pathologist using hematoxylin and eosin stained sections as a guide. Each marked area was dissected from the formalin fixed paraffin-embedded (FFPE) tumor block and transferred into Eppendorf tubes. All samples were digested with proteinase K (Sigma, St. Louis, MO, USA) for more than 24 hours at 56℃ and DNA was then isolated from the digested tissue using a Tissue SV Mini kit (General Biosystem Inc., Seoul, Korea).

Fluorescence in situ hybridization

To evaluate both RET, ALK and PPARγ rearrangement in BRAF-WT PTC, we performed fluorescence in situ hybridization (FISH) analysis in FFPE tumor tissues using the Repeat-Free Poseidon RET (10q11) break-apart probe (Kreatech Diagnostics, Amsterdam, The Netherlands), Vysis ALK break apart probe (Abbott Molecular), and PPARγ (3p25) break probe (Kreatech Diagnostics, Amsterdam, The Netherlands), respectively. These commercially-available probes are designed as a dual-color probe where the two regions across the break-point. The FISH procedures were performed

according to the manufacturer's protocols with modification designed in our laboratory.

For microscopic evaluation, at least 100 intact and nonoverlapping cell nuclei were scored for the presence of a split signal using a Zeiss Axio Imager with appropriate filters. Pictures were captured using a digital microscope camera ProgRes® MF (Jenoptik, Germany) and analyzed with the Isis software (MetaSystems, Germany). The signal pattern interpretation was as follows: interphase nucleus with two co-localized green/red fusion signals identified normal chromosomes, while a separated red and green signals and green/red fusion signals indicated rearranged gene. The positive threshold was defined as more than 10% of signals split and/or isolated red signal in 100 tumor cells.

Immunohistochemistry

We performed IHC staining in FFPE tissue microarray sections of PTC that were 4 μm thick using an automated immunostainer (Leica Microsystems, Milton Keynes, UK). Briefly, the slides were heated for 20 min at 100℃ in Epitope retrieval solution, pH 9.0 (Leica Microsystems). The slides were then

incubated with a monoclonal mouse anti-human ALK antibody (Novocastra, Newcastle Upon Tyne, UK) at a dilution of 1:25. This antibody was raised against a C-terminal portion of the tyrosine kinase domain of ALK and was intended for the qualitative identification of ALK molecules in paraffin sections by light microscopy. Staining intensity was scored as 0 (no staining), 1+ (weak cytoplasmic staining without any background staining), and 2+ (strong cytoplasmic staining). Tumors with 1+ or 2+ expression in more than 10% of the tumor cells were deemed positive for ALK protein expression.

Direct sequencing

Mutations of the NRAS, HRAS, and KRAS point mutations in BRAF-WT PTC and FTC and TERT promoter mutations in PTC and FTC were examined using PCR and amplified using appropriate primers (Table 1). DNA was used as template after quantification by Nano-Drop (Thermo scientific, Wilmington, DE). PCR was performed using a BioMix kit (Bioline, Taunton, MA). Each PCR reaction (50 μL) contained 10 pmol/μL primer set with 1X PCR buffer, 2.5mM MgCl2, and 1μg genomic DNA. PCR was performed under the

following amplification conditions: 94°C for 5 min followed by 50 cycles of 94°C for 1 min, 62°C for 30 s and 72°C for 30 s, and final extension at 72°C for 15 min. Purified PCR products obtained using a QIAquick Gel Extraction kit (Qiagen, Düsseldorf, Germany) were used for sequencing with a Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA). The thermal cycler conditions were as follows: 96°C for 5 min followed by 24 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min, and final extension at 72°C for 5 min. The sequences were analyzed using ABI Prism 3730 Genetic Analyzer (Applied Biosystems), and RAS and TERT mutations were thereby identified. Each DNA sample was assayed at least twice in order to confirm the RAS and TERT mutation status.

Statistical analysis

Chi-square or Fisher's exact test was used to estimate the differences between two groups, with $p<0.05$ considered to indicate significance. All numeric data were expressed as mean ± SD. Continuous variables were compared using a Student *t* test. Kaplan-Meier survival curves with log-rank tests and Cox

proportional hazards regression analyses, censoring patients at the time of recurrence or, if no recurrence, at the time of last follow-up visit, were used to compare recurrence-free survival rates by mutation status. Independent associations of mutations with PTC recurrence were examined by Cox regression analyses. Statistical analyses were performed using the SPSS software (version 20.0, IBM Co., Armonk, NY, USA).

Table 1. Nucleotide sequences of primers used for direct sequencing

Target	primers	Nucleotide sequence
KRAS 12/13	Forward	AAGCGTCGATGGAGGATTT
	Reverse	TGTATCAAAGAATGGTCCTGCA
KRAS 61	Forward	CGTCATCTTTGGAGCAGGAA
	Reverse	ACTCCACTGCTCTAATCCCC
NRAS 12/13	Forward	TACTGTACATGTGGCTCGCC
	Reverse	CCGACAAGTGAGAGACAGGA
NRAS 61	Forward	CCAGATAGGCAGAAATGGGC
	Reverse	CCTTCGCCTGTCCTCATGT
HRAS 12.13	Forward	CAGTCCTTGCTGCCTGGC
	Reverse	CTCCCTGGTACCTCTCATGC
HRAS 61	Forward	GCATGAGAGGTACCAGGGAG
	Reverse	TGATGGCAAACACACACAGG
TERT	Forward	CCCTTCACCTTCCAGCTC
	Reverse	CAGCGCTGCCTGAAACTC

Results

I. Frequency of genetic mutation in PTC

Clinical and pathological features of BRAF wild type PTC

The clinical and pathologic characteristics of BRAF-wild type PTC during the two studied time periods are provided in Table 2. The mean age at diagnosis and sex ratio were not different between two periods. The mean tumor size was significantly smaller in 2009 to 2012 patients than that in 1995 to 2003 ($p < 0.001$) and the proportion of papillary thyroid microcarcinoma (tumor size is less than 1cm) increased from 30.5% in 1995 to 2003 to 54.6% in 2009 to 2012 patients ($p < 0.001$). The frequency of extrathyroidal extension (ETE) was higher in 1995 to 2003 patients ($p = 0.023$). The mean dose of RAI treatment were significantly higher in 1995 to 2003 patients ($p = 0.001$). There were no significant differences for the type of histologic variant, frequency of multifocality and lymph node metastases between two periods.

Frequency of RAS mutations in BRAF-wild type PTC

Among 278 patients with BRAF-wild type PTC, 24 patients (8.6%) had RAS mutations. In 1997 to 2003, RAS mutations were found in 2 of 39 patients (5.1%), whereas 22 of 239 patients (9.2%) had RAS mutations in 2009 to 2012. In 1997 to 2003, all two RAS mutations were NRAS codon 61 mutations. In 2009 to 2012, NRAS codon 61 mutations (n=18, 7.1%) were the most common point mutation (Table 3). KRAS codon 12/13, HRAS 61, and NRAS codon 60 mutations were found in 2, 1, and 1 patients, respectively. A novel mutation in NRAS codon 60 (c.179G>A, Gly60 Glu) was found (Figure 1). A NRAS codon 12/13, a KRAS codon 61 and a HRAS codon 12/13 were not found.

Since the rates of BRAF V600E mutation were reported in a previous data of our institution, the frequency of RAS mutation among all type PTCs could be calculated using those data (61). The rates of RAS mutation among all PTC were estimated at 2.1% in 1997 to 2004 and 2.4% in 2009 to 2012, respectively (Table 4). Those low frequencies of RAS mutation have not been changed over time.

Frequency of RET rearrangements in BRAF WT PTC

RET rearrangements occurred in 1 of 22 BRAF wild-type PTCs (4.5%) in 1997 to 2003 and 15 of 171 (8.8%) in 2009 to 2012, respectively (Figure 2). Among all PTCs, estimated rates were 1.8% in 1997 to 2003 and 2.3% in 2009 to 2012 (Table 4) by using the previously reported data of our institution (61). Patients with RET-rearranged tumors are all females. Among them, 3 cases are follicular variant PTC and 1 case was solid variant PTC (Table 5). Among these patients, no patients had the history of irradiation treatment due to other disease. To evaluate the characteristics of RET rearranged PTC, we compared the clinical and pathologic features between RET-rearranged 15 tumors and normal 156 tumors in 2009 to 2012 (Table 6). There were no significant differences of age, sex, tumor size, multifocality, ETE and lymph node metastasis between two groups.

Frequency of ALK rearrangements in PTC

Among 340 PTC including 79 BRAF V600E PTC, 4 cases showed cytoplasmic stainings for ALK by IHC (Figure 3A). Since there were no

ALK-positive case among BRAF V600E PTC by IHC, we performed FISH analysis for ALK break on only 218 BRAF-wild type PTCs including the four samples (1.8%) that were positive for ALK by IHC to validate for ALK rearrangement. Finally, the results showed break apart signals for just only these 4 samples, corroborating the results for IHC (Figure 3B).

In this series of patients with PTC with ALK rearrangements, a distinct preference in terms of demographic factors or histological subtypes was not found (Table 7). All patients denied having a history of neck irradiation. All four patients with rearrangement of ALK showed favorable responses to the initial surgery with or without radioactive iodine ablation therapy.

Frequency of TERT promoter and BRAF V600E mutation in PTC

TERT C228T and C250T mutations were examined in 433 cases of PTC, and total TERT mutation was found in 18 cases (4.2%), fifteen C228T mutations (3.5%) and three C250T mutations (0.7%), respectively. There was no significant association of TERT with the BRAF mutation. Specifically, on the overall analysis of all PTCs, TERT mutation was found in 6 of 181 (3.3%)

BRAF mutation-negative cases versus 12 of 252 (4.8%) BRAF mutation-positive cases, and conversely, the BRAF mutations were found in 240 of 415 (57.8%) TERT mutation-negative cases versus 12 of 18 (66.7%) TERT mutation-positive cases (odds ratio [OR] 1.46; 95% CI, 0.54 to 3.96; p=0.457).

II. Frequency of genetic mutation in FTC

Frequency of RAS mutations in FTC

Baseline characteristics of the 174 FTC patients in our study are provided in Table 8. Mean age, sex ratio, mean tumor size, and the proportion of widely invasive FTC were not different between two periods. Metachronous pulmonary metastases were found in 1 patient. More patients received the RAI treatments in 2009 to 2012 patients (60% vs 35.3%, p = 0.002). The frequency of recurrence or distant metastases was not statistically different between two periods.

Among 148 patients with FTC, 44 patients (29.7%) had RAS mutations (Table 9). The NRAS codon 61 mutation (n=34, 23.0%) was the most common point mutation. HRAS codon 61 and KRAS codon 61 were found in

9 and 1 patients, respectively. The point mutations identified in our study are summarized in Table 8. In contrast, eight out of 44 follicular adenoma (18.2%) harbored RAS mutations, and among them NRAS codon 61 mutation was the most common point mutation (13.6%).

Frequency of PPARγ rearrangements in FTC

There was no PPARγ rearrangement in BRAF wild type PTC, even in follicular variant PTC. However, 2 PPARγ rearrangements were found in FTC (Figure 4). One patient was an 11.3 years-old-girl at diagnosis, and the tumor size was 5.5cm. Another patient was male and 39.1 years at diagnosis. His tumor size was 8.0 cm. Both patients received RAI treatment after surgery, but no recurrence were found in both of them.

Frequency of TERT promoter and RAS mutation in FTC

TERT C228T and C250T mutations were examined in 120 cases of FTC, and total TERT mutation was found in 7 cases (5.8%), six C228T mutations (5.0%) and one C250T mutation (0.8%). A significant association of TERT with the

RAS mutation was observed. Specifically, on the overall analysis of all FTCs, TERT mutation was found in 2 of 80 (2.5%) RAS mutation-negative cases versus 5 of 40 (12.5%) RAS mutation-positive cases, and conversely, the RAS mutation was found in 35 of 113 (31.0%) TERT mutation-negative cases versus 5 of 7 (71.4%) TERT mutation-positive cases (OR 5.57; 95% CI, 1.03 to 30.12; p=0.04).

III. Clinical implication of genetic mutations in PTC and FTC

Relationship of RAS mutations with clinicopathologic outcomes of PTC

Since there were only 2 RAS mutant patients in 1997 to 2003 patients, we compared the clinicopathologic features according to RAS mutation status only in 2009 to 2012 patients (Table 10). The presence of a RAS mutation was significantly associated with tumor size, type of variant, ETE, and LN metastases. Mean tumor size was larger (1.9 ± 1.5 cm vs 1.1 ± 0.8 cm, p = 0.015) but LN metastases were lower in RAS mutant tumor than those in RAS-WT tumor (4.5% vs 58.1% p = 0.007), since there were more follicular variant PTCs in RAS mutant tumor (63.6% vs 13.0%, $p < 0.001$). There was

no difference in the recurrence rates.

Impacts of TERT mutation on clinicopathologic outcome of PTC

TERT mutation was found to be significantly associated with several high-risk clinicopathologic characteristics, including older patient age, larger tumor size, and high-risk stage (Table 11). Tumor recurrence was 41 of 373 (11.0%) in TERT mutations-negative cases versus 6 of 18 (33.3%) in TERT mutation-positive cases (hazard ratio [HR] 3.38; 95% CI 1.87 to 8.42; $p = 0.005$; Table 12). The HR of TERT mutation for tumor recurrence remained significant after adjustment for patient age and sex (HR 5.82; 95% CI 2.29 to 14.79; $p < 0.001$), but they lost significance with the 95% CI marginally crossing 1.0 after additional adjustment for aggressive tumor behaviors including tumor size, extrathyroidal extension and lymph node metastases (HR 2.68; 95% CI 1.01 to 6.25; $p = 0.047$). However there were no differences in tumor recurrence between BRAF negative cases and BRAF positive cases (HR 0.63; 95% CI 0.35 to 1.14; $p = 0.129$).

We performed Kaplan Meier and log-rank analyses of disease-free survival rates of patients by genotype. In analyses of all PTCs, tumor disease-free survival curves had a modest decline in patients negative for TERT (Figure 5A) or BRAF V600E (Figure 5B). They declined further with TERT mutation (Figure 5A) but not with BRAF (Figure 5B). Figure 6 shows the impacts of individual BRAF or TERT mutations or their coexistence on tumor recurrence-free survival curves of all patients with PTC, but there was no influence of coexisting those two mutations on disease-free survival in PTC.

Relationship of RAS mutations with clinicopathologic outcomes of PTC

When we compared clinicopathologic features of FTC according to RAS mutation status (Table 13), distant metastases were more common in RAS mutant group (9.1% vs 1.8%), which was statistically insignificant (p = 0.066). Other clinicopathological features showed no statistical differences between RAS-wild type and RAS mutant tumors.

Impacts of TERT mutation on clinicopathologic outcome of FTC

TERT mutation was found to be significantly associated with several high-risk clinicopathologic characteristics, including larger tumor size and high-risk stage (Table 14). Tumor recurrence was 6 of 109 (5.5%) in TERT mutations-negative cases versus 2 of 7 (28.6%) in TERT mutation-positive cases (HR 7.22; 95% CI 1.39 to 37.45; p = 0.019; Table 15). The HR of TERT mutation for tumor recurrence remained significant after adjustment for patient age and sex (HR 6.93; 95% CI 1.13 to 42.38; p = 0.036), but they lost significance after additional adjustment for aggressive tumor behaviors including tumor size, extrathyroidal extension and lymph node metastases (HR 1.06; 95% CI 0.002 to 453.38; p = 0.985). However there were no differences in tumor recurrence between RAS negative cases and RAS positive cases (HR 2.65; 95% CI 0.59 to 11.86; p = 0.203).

We performed Kaplan Meier and log-rank analyses of disease-free survival rates of patients by genotype. In analyses of all FTCs, tumor disease-free survival curves had a modest decline in patients negative for TERT (Figure 7A) or RAS mutation (Figure 7B). They declined further with TERT

mutation (Figure 7A) but not with RAS (Figure 7B). Figure 8 shows the impacts of individual RAS or TERT mutations or their coexistence on tumor recurrence-free survival curves of all patients with FTC, but there was no influence of coexisting those two mutations on disease-free survival in FTC.

Table 2. Trends in clinical and pathologic characteristics of BRAF-wild type PTC over time, Seoul National University Hospital 1997-2012

characteristics	Time period		*p* value
	1997-2004 (n=59)	2009-2012 (n=295)	
Age (years)	45.2 ± 14.8	45.5 ± 13.2	0.869
Sex (F:M)	54:5 (91.2%)	266:29 (90.2%)	0.486
Tumor size			<0.001
≤1.0cm	18 (30.5%)	160 (54.6)	
1.1-2.0cm	18 (30.5%)	102 (34.8%)	
2.1-3.0cm	12 (20.3%)	22 (7.5%)	
3.1-4.0cm	6 (10.2%)	4 (1.4%)	
>4.0cm	5 (8.5%)	5 (1.7%)	
Histologic variant			0.191
Classic papillary	47 (79.7%)	220 (75.1%)	
Follicular	11 (18.6%)	54 (18.4%)	
Others	1 (1.7%)	19 (6.4%)	
Multifocality	21 (36.8%)	108 (36.3%)	0.546
Lymph node metastasis	23 (48.9%)	108 (36.3%)	0.533
Extrathyroidal extension	34 (57.6%)	144 (48.8%)	0.023
RAS mutation	2/39 (5.1%)	22/239 (9.2%)	0.548
Classic	0/27 (0.0%)	8/180 (4.4%)	
Follicular	2/11 (18.2%)	14/42 (33.3%)	
RET rearrangement	1/22 (4.5%)	15/182 (8.2%)	
TNM Stage			0.078
I-II	35 (59.3%)	206 (69.8%)	
III-IV	24 (40.7%)	89 (30.2%)	
RAI treatment (mCi)	140 ± 216	60 ± 96	0.001
Recurrence	4 (8.7%)	22 (8.5%)	1.000
Distant metastases	2 (4.3%)	3 (1.1%)	0.167

Abbreviations: PTC, papillary thyroid carcinoma; RAI, radioactive iodine

Table 3. The frequency of RAS mutations in papillary thyroid cancer (2009-2012)

RAS mutation		Time periods	
		1997-2003 (n=39)	2009-2012 (n=239)
NRAS codon 61	Q61R	2 (5.1%)	17 (7.1%)
	Q61K	0	1 (0.4%)
NRAS codon 60	G60E	0	1 (0.4%)
KRAS codon 12/13	G12V	0	1 (0.4%)
	G12R	0	1 (0.4%)
HRAS codon 61	Q61R	0	1 (0.4%)

Table 4. Changes in prevalence of somatic mutations in all type PTC over time

Mutation	1997-2004	2009-2012
BRAF, n (%) *	112/189 (59.2)	1792/2431 (73.7)
RAS, n (%) **	2/39 (2.1)	22/239 (2.4)
HRAS	0/39 (0.0)	1/239 (0.1)
NRAS	2/39 (2.1)	19/239 (2.1)
KRAS	0/39 (0.0)	2/239 (0.1)
RET/PTC, n (%) **	1/22(1.8)	15/171 (2.3)

* Data from Hong AR, Endocrinolgy and Metabolism 2014

** Among all PTC (including BRAF V600E PTC)

Abbreviaion: PTC, papillary thyroid carcinoma

Table 5. Clinicopathologic features of 15 RET rearranged patients

No	Sex /Age	Pathologic diagnosis	Tumor Size (mm)	Multi focal ity	Co-existing Hashi -moto's thyroiditis	pTNM	Cell% with split RET signal
1	51/F	cPTC	12	No	Yes	T1N0M0	37
2	28/F	fvPTC	30	Yes	No	T3N1M0	56
3	53/F	cPTC	16	No	No	T3N1M0	17
4	44/F	cPTC	12	Yes	Yes	T1N0M0	36
5	29/F	cPTC	8	No	Yes	T3N0M0	52
6	52/F	cPTC	11	No	Yes	T1N0M0	10
7	52/F	cPTC	16	Yes	Yes	T3N1M0	32
8	22/F	cPTC	12	No	Yes	T3N1M0	33
9	41/F	svPTC	11	No	Yes	T3N0M0	26
10	51/F	fvPTC	7	No	No	T3N0M0	12
11	56/F	cPTC	14	No	No	T3N1M0	51
12	48/F	cPTC	11	Yes	Yes	T3N0M0	31
13	31/F	cPTC	8	No	No	T3N1M0	53
14	30/F	cPTC	7	No	No	T1N1M0	34
15	22/F	fvPTC	7	No	No	T1N0M0	48

Abbreviations: cPTC, classic papillary thyroid carcinoma; fvPTC, follicular variant papillary thyroid carcinoma; svPTC, solid variant papillary thyroid carcinoma

Table 6. Comparisons between RET rearrangement and clinicopathological features (2009-2012)

Parameter	RET rearrangement Negative (N=156)	RET rearrangement Positive (N=15)	P value
Age (years)	40(21-56)	45(13-82)	0.139
Sex (F:M)	140:16	15:0	0.366
Mean tumor size (cm)	1.2±0.8	1.2±0.3	0.977
Diameter≤1cm	74(47.4%)	5(33.3%)	0.418
Variant			0.998
Classic type	123(73.2%)	11 (73.3%)	
Follicular variant	40(23.8%)	3 (20%)	
Multifocality	57(36.5%)	5 (33.3%)	0.690
ETE	83(53.2%)	10(66.7%)	0.380
LN metastasis	73(46.8%)	7 (46.7%)	0.629
Stage (AJCC 2010)			1.000
I-II	101(64.7%)	10(66.7%)	
III-IV	55(35.3%)	5(33.3%)	
Coexisting Hashimoto's thyroiditis	73(46.8%)	8(53.3%)	0.788
RAI treatment	105(62.1%)	10(66.7%)	0.728
Recurrence	4(2.4%)	1(6.7%)	0.285
Distant metastases	0 (0%)	1 (6.7%)	0.073

Abbreviations: PTC, papillary thyroid carcinoma; LN, lymph node; ETE, extrathyroidal extension; RAI, radioactive iodine

Table 7. Clinicopathologic features of the four patients with PTC with rearrangement of ALK

	Patient 1	Patient 2	Patient 3	Patient 4
Age (yrs)	13	50	36	48
Sex (F:M)	Female	Female	Male	Female
History of neck irradiation	-	-	-	-
Tumor size	2.8	0.6	1.9	0.7
Variant	Solid	Follicular	Classic	Classic
ETE	microscopic	microscopic	microscopic	-
LNM	-	-	+	+
Coexisting pathology	Lymphocytic thyroiditis	Lymphocytic thyroiditis	Lymphocytic thyroiditis	Lymphocytic thyroiditis
TNM staging	I	III	I	III
RAI	ND	ablated	ablated	ablated
Recurrence	no	no	no	no

Abbreviations: PTC, papillary thyroid carcinoma; ETE, extrathyroidal extension; LNM, lymph node metastases; RAI, radioactive iodine; ND, not done

Table 8. Trends in clinical and pathologic characteristics of FTC over time, Seoul National University Hospital 1997-2012

	Time period		*p* value
	1997-2004 (n=93)	2009-2012 (n=81)	
Age (years)	48.8 ± 14.4	45 ± 14.3	0.133
Sex (F:M)	74:19	60:21	0.248
Tumor			
Mean size ± SD (cm)	3.5 ± 1.7	3.4 ± 1.9	0.662
Widely invasive FTC	8 (8.6%)	5 (6.2%)	0.811
Lymph node metastases	4 (4.4%)	1 (1.8%)	0.386
Distant metastases	5 (5.5%)	3 (3.7%)	0.426
RAS mutation	28/79 (35.4%)	16/69 (23.3%)	0.073
PPARγ rearrangement	N/A	2/61 (3.3%)	N/A
RAI treatment	24/68 (35.3%)	48/80 (60%)	0.002
Recurrence	7 (9.1%)	2 (2.5%)	0.077

Abbreviations: FTC, follicular thyroid carcinoma; RAI, radioactive iodine; N/A, not applicable

Table 9. The frequency of RAS mutations in study groups

	Total (N=470)	FA (N=44)	FTC (N=148)	PTC (N=278)
Any RAS mutation (%)	76 (16.2)	8 (18.2)	44 (29.7)	24 (8.6)
NRAS codon 61 (%)	60 (12.8)	6 (13.6)	34 (23.0)	20 (7.2)
HRAS codon 61 (%)	44 (9.4)	1 (2.3)	9 (6.1)	1 (0.4)
KRAS codon 61 (%)	2 (4.3)	1 (2.3)	1(0.7)	0
KRAS codon 12/13 (%)	2 (4.3)	0	0	2 (0.7)
HRAS codon 12/13 (%)	0	0	0	0
KRAS codon 12/13 (%)	0	0	0	0

Abbreviations: FA, follicular adenoma; FTC, follicular thyroid carcinoma; PTC, papillary thyroid carcinoma

Table 10. Comparisons of clinicopathological features in BRAF wild type PTC patients according to RAS mutation status (2009-2012)

Parameter	RAS wild type (N=217)	RAS mutant (N=22)	*p* value
Age (years)	45.3 ± 13.2	50.0 ± 12.5	0.094
Sex (F:M)	201:16	17:5	0.031
Mean tumor size (cm)	1.1 ± 0.8	1.9 ± 1.5	0.015
Diameter≤1cm	123 (57.2%)	7 (31.8%)	<0.001
Variant			<0.001
Classic type	172 (80.0%)	8 (36.4%)	
Follicular variant	28 (13.0%)	14 (63.6%)	
Multifocality	72 (33.2%)	9 (40.9%)	0.724
Extrathyroidal extension	106 (47.5%)	6 (27.3%)	0.184
Lymph node metastasis	126 (58.1%)	1 (4.5%)	0.007
TNM Stage			0.326
I-II	151 (69.6%)	18 (81.8%)	
III-IV	66 (30.4%)	4 (18.2%)	
RAI treatment	120 (55.3%)	12 (54.5%)	0.504
Recurrence	19 (9.8%)	1 (5.6%)	1.000
Distant metastases	2 (1.0%)	1 (5.6%)	0.235

Abbreviations: PTC, papillary thyroid carcinoma; RAI, radioactive iodine

Table 11. Comparisons of clinicopathological features in PTC patients according to TERT mutation status

	TERT wild type (N=415)	TERT mutation (N=18)	*p* value
TERT mutation type			
C228T	-	15 (3.5%)	
C250T	-	3 (0.7%)	
Age (years)	45 ± 13	57 ± 13	<0.001
Sex (F:M)	370:45	15:3	0.489
Tumor size (cm)	1.5 ± 1.2	2.6 ± 1.5	0.005
Extrathyroidal extension	248 (59.8%)	14 (77.8%)	0.146
Microscopic	135 (32.5%)	3 (16.7%)	
Gross	113 (27.2%)	11 (61.1%)	
Lymph node metastasis	146 (37.4%)	10 (55.6%)	0.112
Distant metastasis	3 (0.7%)	4 (22.2%)	<0.001
RAI dose (mCi)	89 ± 178	201 ± 234	0.059
ATA risk of recurrence			<0.001
Low	127 (30.6%)	2 (11.1%)	
Intermediate	157 (37.8%)	2 (11.1%)	
High	131 (31.6%)	14 (77.8%)	
TNM stage			<0.001
I-II	319 (76.9%)	5 (27.8%)	
III-IV	96 (23.1%)	13 (72.2%)	
Recurrence/Persistence	41 (11.0%)	6 (33.3%)	0.004

Abbreviations: PTC, papillary thyroid carcinoma; RAI, radioactive iodine; ATA, American Thyroid Association

Table 12. Association of TERT or BRAF V600E mutation with PTC recurrence

Mutation status	Recurrence N (%)	Unadjusted			Adjusted*			Adjusted†		
		HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value
TERT (-)	41/373 (11.0%)	1			1			1		
TERT (+)	6/18 (33.3%)	3.38	1.43-7.97	0.005	5.82	2.29-14.79	<.001	2.68	0.94-7.64	0.065
BRAF (-)	20/160 (12.5%)	1			1			1		
BRAF (+)	27/231 (11.7%)	0.63	0.35-1.14	0.129	0.65	0.36-1.17	0.150	0.69	0.37-1.32	0.262

Abbreviations: PTC, papillary thyroid carcinoma; HR, hazard ratio

*Adjustment was made for patient age at diagnosis and sex.

† Adjustment was made for patient age at diagnosis, sex, tumor size, extrathyroidal extension, and lymph node metastasis

Table 13. Comparisons of clinicopathological features in FTC patients according to RAS mutation status over time

	RAS wild type (n=104)	RAS mutant (n=44)	*p* value
Age (years)	47.4 ± 14.6	46.1 ± 12.6	0.586
Sex (F:M)	80:24	34:10	0.572
Tumor			
Mean size±SD (cm)	3.6 ± 1.9	3.3 ± 1.4	0.281
Widely invasive FTC	8 (14.0%)	2 (11.1%)	0.453
Lymph node metastases	1 (1.3%)	2 (5.6%)	0.234
Distant metastases	2 (1.9%)	4 (9.1%)	0.066
RAI treatment	46 (47.9%)	17 (45.9%)	0.497
Recurrence	4 (3.1%)	4 (9.5%)	0.120

Abbreviations: FTC, follicular thyroid carcinoma; RAI, radioactive iodine; N/A, not applicable

Table 14. Comparisons of clinicopathological features in FTC patients according to TERT mutation status

	TERT wild type (N=113)	TERT mutation (N=7)	*p* value
TERT mutation type			
C228T	-	6 (5.0%)	
C250T	-	1 (0.8%)	
Age (years)	45 ± 14	55 ± 13	0.060
Sex (F:M)	91:22	4:3	0.157
Tumor size (cm)	3.5 ± 1.8	5.2 ± 2.3	0.018
Extrathyroidal extension	68 (60.2%)	5 (71.4%)	0.703
Microscopic	41 (36.3%)	4 (57.1%)	
Gross	27 (23.9%)	1 (14.3%)	
Lymph node metastasis	1 (1.2%)	1 (33.3%)	0.067
Distant metastasis	5 (4.4%)	1 (14.3%)	0.308
RAI dose (mCi)	112 ± 296	153 ± 185	0.722
ATA risk of recurrence			0.874
Low	43 (38.1%)	2 (28.6%)	
Intermediate	40 (35.4%)	3 (42.9%)	
High	30 (26.5%)	2 (28.6%)	
TNM stage			0.011
I-II	88 (77.9%)	2 (28.6%)	
III-IV	25 (22.1%)	5 (71.4%)	
Recurrence/Persistence	6 (5.5%)	2 (28.6%)	0.074

Abbreviations: FTC, follicular thyroid carcinoma; RAI, radioactive iodine; ATA, American Thyroid Association

Table 15. Association of TERT or RAS mutation with FTC recurrence

Mutation status	Recurrence N (%)	Unadjusted			Adjusted*			Adjusted†		
		HR	95% CI	*p* value	HR	95% CI	*p* value	HR	95% CI	*p* value
TERT (-)	6/109 (5.5%)	1			1			1		
TERT (+)	2/7 (28.6%)	7.22	1.39-37.45	0.019	6.93	1.13-42.38	0.036	1.06	0.002-453.38	0.985
RAS (-)	3/77 (3.9%)	1			1			1		
RAS (+)	5/39 (28.6%)	2.65	0.59-11.86	0.203	4.10	0.87-19.33	0.075	11.44	0.79-165.28	0.074

Abbreviations: FTC, follicular thyroid carcinoma; HR, hazard ratio

*Adjustment was made for patient age at diagnosis and sex.

† Adjustment was made for patient age at diagnosis, sex, tumor size, extrathyroidal extension, and lymph node metastasis.

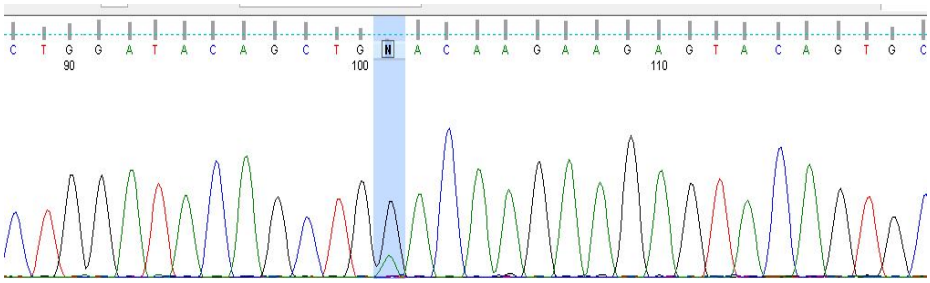


Figure 1. c.179G>A, Gly60 Glu in BRAF wild type papillary thyroid cancer

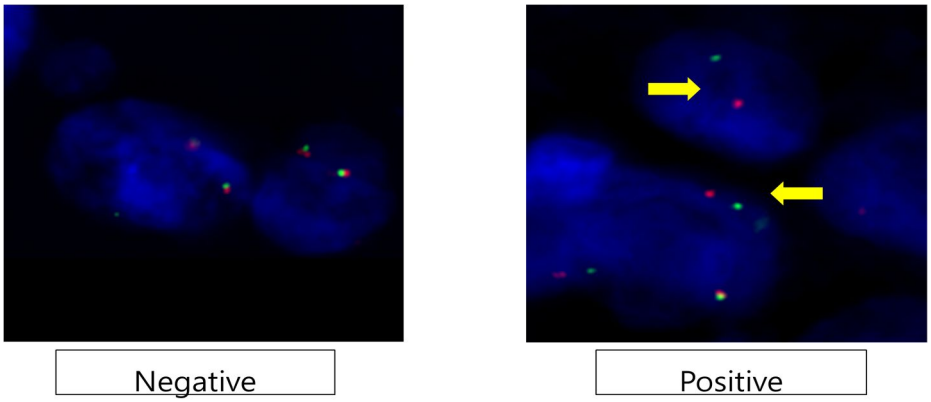


Figure 2. Interphase fluorescence in situ hybridization (FISH) analysis demonstrating the RET rearrangement of chromosome 10. Yellow arrows indicate the split red and green signals that are indicative of RET rearrangement.

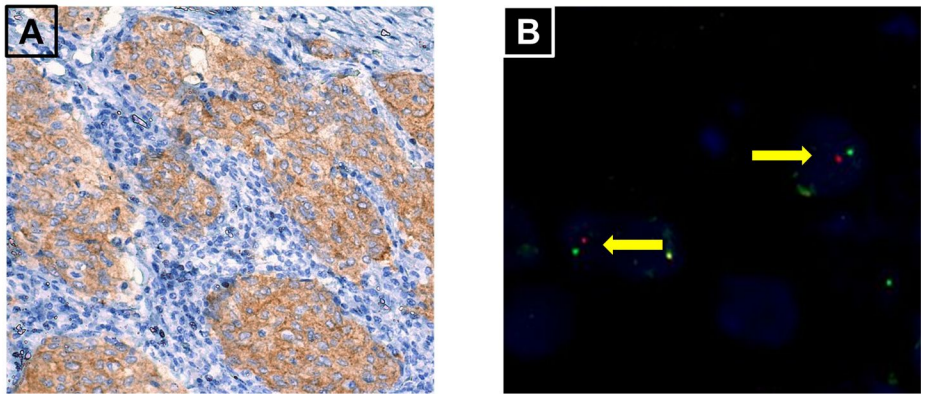


Figure 3. (A) Photomicrograph image of IHC using the anti-ALK antibody showing strong cytoplasmic staining (magnification: 200x). Confirmation of ALK rearrangement by FISH (B) using the break apart ALK probe to show splitting of one pair of red and green signals (yellow arrows)

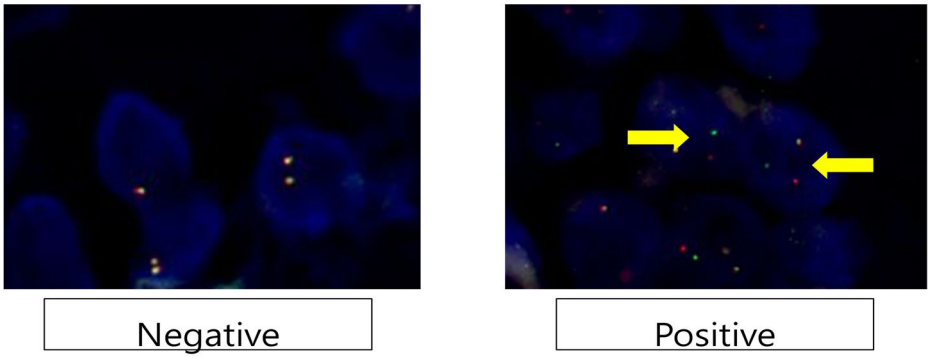
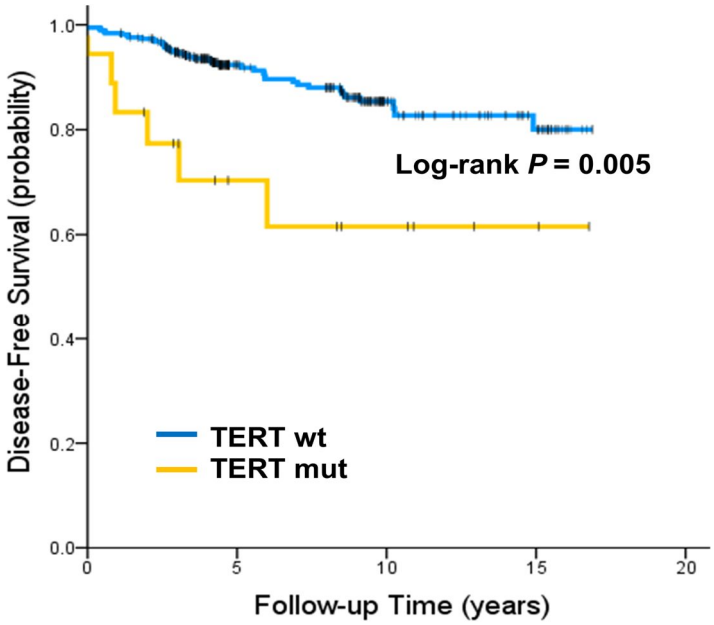


Figure 4. Interphase fluorescence in situ hybridization (FISH) analysis demonstrating the PPARγ rearrangement of chromosome 3. Yellow arrows indicate the split red and green signals that are indicative of PPARγ rearrangement.

A.



B.

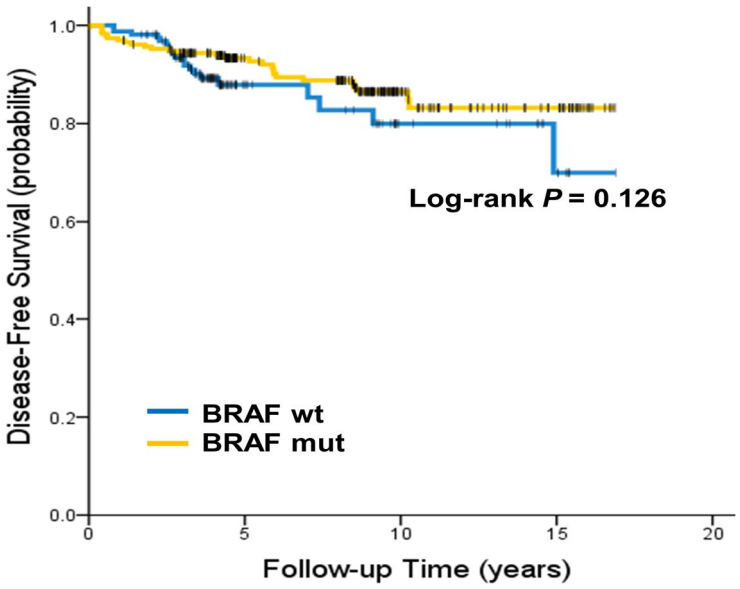


Figure 5. Kaplan-Meier analyses of the impact of TERT (A) and BRAF V600E (B) mutation on disease-free survival of patients with papillary thyroid cancer

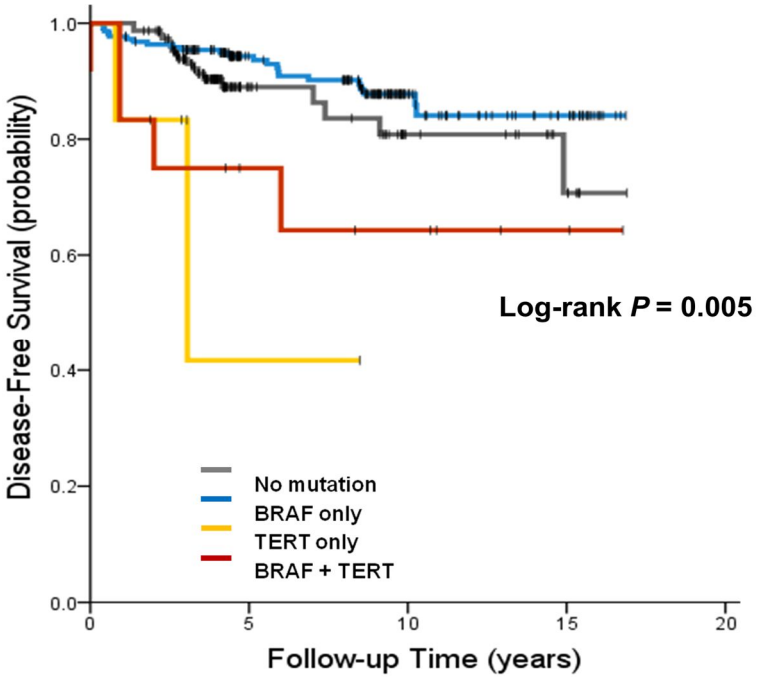
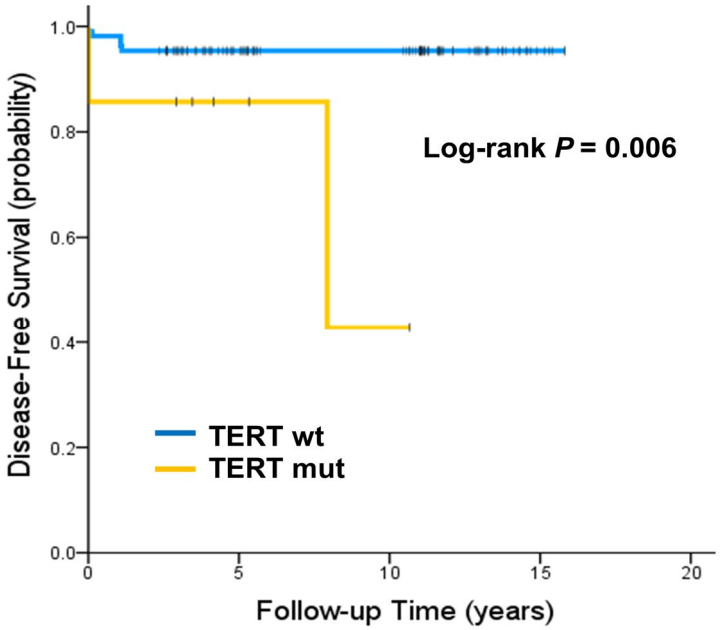


Figure 6. Kaplan-Meier analyses of the impacts of BRAF or TERT alone or their coexistence of disease-free survival of patients with papillary thyroid cancer

A.



B.

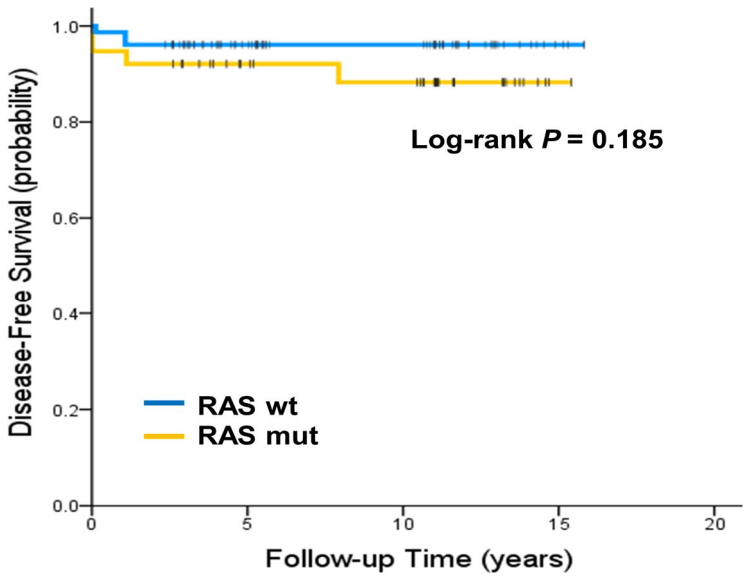


Figure 7. Kaplan-Meier analyses of the impact of TERT (A) and RAS (B) mutation on disease-free survival of patients with follicular thyroid cancer

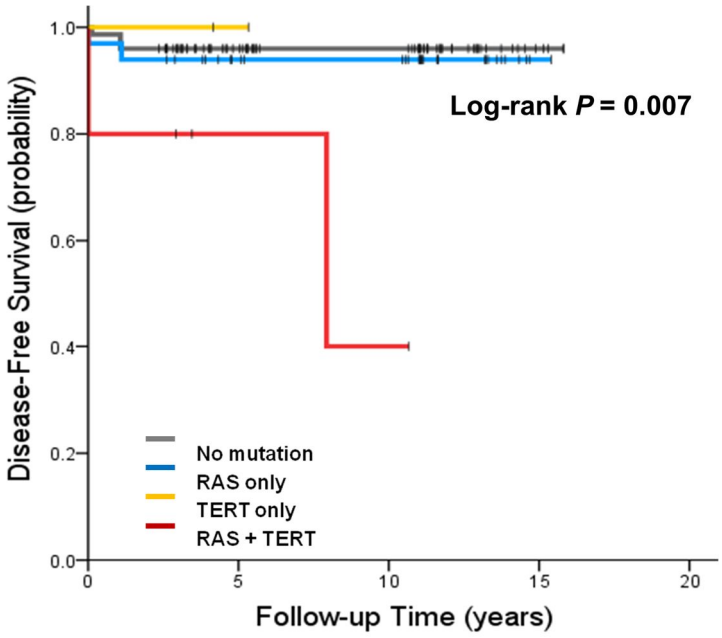


Figure 8. Kaplan-Meier analyses of the impacts of RAS or TERT alone or their coexistence of disease-free survival of patients with follicular thyroid cancer

Discussion

In this study, analysis for genetic mutation of Korean thyroid cancers was performed by various methods using direct sequencing, IHC and FISH methods. Since prevalence and its aggressiveness of BRAF mutation in PTCs are well-known, we focused the other genetic mutations including RAS mutation, RET, ALK, and PPARγ rearrangements or TERT mutation. Also, we evaluated whether genetic mutational profiling of Korean thyroid cancer have changed over 10 years.

Although differentiated thyroid cancers have good prognosis, they can recur up to 10~20% of all (65). It is suggested that poor prognostic factors for thyroid cancer are aggressive histology such as ETE or LN metastases, postoperative serum thyroglobulin or having an aggressive genetic mutation (66, 67). For example, several studies have reported that BRAF mutation is an independent prognostic vale for recurrence in PTC (68). Thus, it is important to evaluate the clinical significance of genetic mutation in thyroid cancer for prediction of prognosis as well as diagnosis of thyroid cancer.

In the present series of 278 BRAF-wild type PTCs, the frequency of RAS mutation was relatively low, showing 24 (8.6%) any RAS mutation of BRAF-wild type PTCs. Among all PTC, the frequencies were estimated at approximately 2.1% in 1997 to 2003 and 2.4% in 2009 to 2012 by using the previous data of our hospital (61). Moreover, the trends in frequency of RAS mutation have not been changed, since in Korea the proportion of follicular variant PTC stands still unlike the United States (59). In the United States, while the overall proportion of BRAF mutations in PTC was stable (approximately 45%), RAS mutations after 2000 were increased (from 3~15% to 25%) with the percentages of the follicular variant histology (59). In our data, RAS mutant tumors are found more in follicular variant PTC, and the proportion of follicular variant PTCs have been maintained low since the most common PTCs in Korea have been classic type PTCs still now.

The frequency of RET rearrangements in this study was similar with those of other countries. 15 patients (8.8%) showed RET rearrangement by FISH analysis of 171 BRAF wild type PTC patients, but the rate of RET rearrangement among all type PTC could be estimated 2.3% by using the

previous data of our institution (61). RET/PTC rearrangement is well known to have a strong association with radiation exposure. As many as 80% of PTCs in persons exposed to radiation either accidentally (mostly radioiodine) or therapeutically (mostly external beam) carry RET/PTC (35-37). In contrast, point mutations of BRAF and RAS are less common in radiation-related tumors (39). For PTCs found in atomic bomb survivors in Japan, the RET/PTC prevalence increased monotonically with increasing radiation dose, whereas BRAF point mutations showed an inverse relation (40, 41). Childhood exposure to external ionizing radiation is a well-established risk factor for thyroid cancer (3). A low frequency of RET rearrangement in Korea over time suggests that the increasing incidence of thyroid cancer was not likely to be due to ionizing radiation exposure, at least in the form of environmental and therapeutic radiation known to be associated with RET/PTC rearrangement. Since Korea is located nearby Japan, we have concerned the radiation effect from Fukushima nuclear disaster occurred in 2012. Subjects of this study is included before July 2012, thus it would be important that RET/PTC rearrangement should be evaluated in Korean PTC

patients diagnosed after Fukushima accident, especially in young patients.

RAS mutation-driven carcinogenesis has been associated with exposure to environmental chemical carcinogens in experimental animals and in human tumors (69-72). Examples of the latter include RAS mutations in hepatocellular carcinomas in workers exposed to vinyl chloride (73), in lung adenocarcinomas associated with tobacco smoking (74), and in acute leukemias in patients with occupational exposure to chemical agents or in their children (75). In this study, the percentage of RAS mutation has not been changed over time unlike other countries (59, 60).

In FTC patients, several studies reported that RAS mutations are correlated with poor prognosis (20, 76). From recent Korean reports, the presence of a RAS mutation, especially a NRAS codon 61 mutation, was significantly associated with the distant metastasis (77). However in this study, RAS mutant tumors were shown to have neither poor prognostic factor nor poor clinical outcome such as distant metastases or recurrence. Previous studies enrolled more metastatic cancer patients than this study, but more patients diagnosed FTC recently were included and that might be the main reason

why RAS mutant tumor did not have poor prognosis in this study.

PPARγ rearrangements were found in two cases (3.3%) of 61 FTC patients, but they were not detected among PTC, even in follicular variant PTC. When first described in 2000, PAX8/PPARγ rearrangement was assumed to be specific for FTC (45). Subsequent studies have confirmed the presence of PAX8/PPARγ rearrangement in 30-35% of FTC and also have found it in 2-13% of benign follicular adenomas (47-49). Additional studies have reported the occurrence of PAX8/PPARγ rearrangement in the follicular variant of PTC, typically with low frequency (1-5%) (78). However, in some reports, PAX8/PPARγ rearrangements were found in a much higher prevalence (37.5%) of follicular variants of PTC (50, 79). As a result, the frequency of PAX8/PPARγ rearrangement in PTC remains controversial until now.

ALK is involved in the initiation and progression of many different cancer types including lymphoma, neuroblastoma, and non-small cell lung cancer (80, 81). Preclinical studies in thyroid cancer patients, indicated that rearrangements of ALK in thyroid cancers may also be sensitive to ALK inhibitors (43). Therefore, it is expected that tumors with rearrangement of

ALK will become a unique, targetable subset of thyroid cancer and that identification of this emerging biomarker will have an effect on the diagnosis and treatment of patients with advanced differentiated thyroid cancer. In this study, PTC with ALK rearrangement invariably expressed an ALK protein that could be readily be identified in the paraffinized samples using IHC with an ALK monoclonoal antibody. As rearrangements of ALK represent a potential therapeutic target and FISH is a validated method for the detection of actual fusions that correlate with the response to treatment, the use of IHC can be recommended as a screening procedure and the use of FISH for the final confirmation of rearrangement of ALK.

Mutation in the promoter of TERT were recently identified as common events in melanomas, glioblastomas, bladder carcinomas, and other tumors (53, 54, 82, 83). TERT mutations are enriched in advanced cancers, such as metastatic melanomas (54) and adult primary glioblastoma (82) with respect to their aggressive counterparts. This study showed that this is also the case in thyroid cancers, and and recent several reports showed compatible results (51, 55, 84). Moreover, coexisting TERT and BRAF V600E mutations were

even more significantly associated with clinicopathological aggressiveness (56, 57). However, those results were not shown in our study, and the possible reasons are that the number of subjects was not enough or that BRAF V600E mutations are more prevalent in Korea than other countries.

Recently, The Cancer Genome Atlas (TCGA) projects were presented the results from a comprehensive multiplatform analysis of 496 PTCs, the largest cohort studies to date (85). They observed a low frequency of somatic alterations (relative to other carcinomas) and extended the set of known PTC driver mutations including diverse gene fusions. These discoveries reduced the fraction of PTC cases with unknown oncogenic driver from 25% to 3.5%. From their data, RAS mutations were found in 12.9% of PTC, RET fusions in 6.8% and TERT mutations in 9.4%. The mutational rates of TCGA data were higher than those of this study in Korea. Remaining unknown genetic alterations of Korean PTCs should be investigated further to inform the management of thyroid cancer better.

This study has several limitations. First, 10 to 20% of tumors had massive calcification in them, so they had insufficient material for mutation analysis.

However, the demographic and clinical features of these patients were generally similar to those of other patients. Second, our case series was derived from one institution, and therefore our results may not be representative of the entire Korea. However, demographic and tumor features (age at diagnosis, sex, and tumor size) of these patients with PTCs were generally consistent with those reported previously in other hospital of Korea. Only cases with an original diagnosis of PTC were selected for this study; consequently, the proportion of follicular variant PTCs, particularly of the encapsulated type that in the early calendar period could have been classified as follicular adenoma or carcinoma, may be underestimated and the trend overestimated.

In conclusion, our results suggests that the prevalence of RAS mutation in Korea was relatively low, due primarily to low proportion of follicular variant in all PTCs unlike other countries. The percentage of RET rearrangements also showed low, suggesting different etiologic factors in Korea, compared with other countries. RAS mutations were shown in 23.4% of FTC, but it was not a poor prognostic factor for clinical outcome in FTC

patients. TERT promoter mutation was associated with aggressive clnicopathologic features and outcome in both PTC and FTC, but its coexistence with BRAF V600E or RAS mutation had no impact on clinical aspects. These differences between Korea and other countries on the mutational profiles and their impact on the outcome and prognosis should be investigated in several ways.

References

1. Chen AY, Jemal A, Ward EM. Increasing incidence of differentiated thyroid cancer in the United States, 1988-2005. Cancer. 2009;115(16):3801-7.

2. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA. 2006;295(18):2164-7.

3. Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, Pottern LM, et al. Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. Radiation Research. 1995;141(3):259-77.

4. Cardis E, Kesminiene A, Ivanov V, Malakhova I, Shibata Y, Khrouch V, et al. Risk of thyroid cancer after exposure to 131I in childhood. Journal of the National Cancer Institute. 2005;97(10):724-32.

5. Mettler FA, Jr., Bhargavan M, Faulkner K, Gilley DB, Gray JE, Ibbott GS, et al. Radiologic and nuclear medicine studies in the United States and worldwide: frequency, radiation dose, and comparison with other radiation sources--1950-2007. Radiology. 2009;253(2):520-31.

6. Nikiforov YE. Is ionizing radiation responsible for the increasing incidence of thyroid cancer? Cancer. 2010;116(7):1626-8.

7. Enewold L, Zhu K, Ron E, Marrogi AJ, Stojadinovic A, Peoples GE, et al. Rising thyroid cancer incidence in the United States by demographic and tumor characteristics, 1980-2005. Cancer Epidemiology, Biomarkers & Prevention. 2009;18(3):784-91.

8. Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA. High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Research. 2003;63(7):1454-7.

9. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nature Reviews Endocrinology. 2011;7(10):569-80.

10. Prior IA, Lewis PD, Mattos C. A comprehensive survey of Ras mutations in cancer. Cancer Research. 2012;72(10):2457-67.

11. Suarez HG, du Villard JA, Severino M, Caillou B, Schlumberger M, Tubiana M, et al. Presence of mutations in all three ras genes in human thyroid tumors. Oncogene. 1990;5(4):565-70.
12. Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. Modern pathology. 2008;21 Suppl 2:S37-43.
13. Ricarte-Filho JC, Ryder M, Chitale DA, Rivera M, Heguy A, Ladanyi M, et al. Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. Cancer Research. 2009;69(11):4885-93.
14. Adeniran AJ, Zhu Z, Gandhi M, Steward DL, Fidler JP, Giordano TJ, et al. Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. The American Journal of Surgical Pathology. 2006;30(2):216-22.
15. Zhu Z, Gandhi M, Nikiforova MN, Fischer AH, Nikiforov YE. Molecular profile and clinical-pathologic features of the follicular variant of papillary thyroid carcinoma. An unusually high prevalence of ras mutations. American Journal of Clinical Pathology. 2003;120(1):71-7.
16. Esapa CT, Johnson SJ, Kendall-Taylor P, Lennard TW, Harris PE. Prevalence of Ras mutations in thyroid neoplasia. Clinical Endocrinology. 1999;50(4):529-35.
17. Motoi N, Sakamoto A, Yamochi T, Horiuchi H, Motoi T, Machinami R. Role of ras mutation in the progression of thyroid carcinoma of follicular epithelial origin. Pathology, Research and Practice. 2000;196(1):1-7.
18. Namba H, Rubin SA, Fagin JA. Point mutations of ras oncogenes are an early event in thyroid tumorigenesis. Mol Endocrinol. 1990;4(10):1474-9.
19. Basolo F, Pisaturo F, Pollina LE, Fontanini G, Elisei R, Molinaro E, et al. N-ras mutation in poorly differentiated thyroid carcinomas: correlation with bone metastases and inverse correlation to thyroglobulin expression. Thyroid. 2000;10(1):19-23.
20. Garcia-Rostan G, Zhao H, Camp RL, Pollan M, Herrero A, Pardo J,

et al. ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. Journal of Clinical Oncology. 2003;21(17):3226-35.

21. Santoro M, Carlomagno F, Hay ID, Herrmann MA, Grieco M, Melillo R, et al. Ret oncogene activation in human thyroid neoplasms is restricted to the papillary cancer subtype. The Journal of Clinical Investigation. 1992;89(5):1517-22.

22. Jhiang SM, Sagartz JE, Tong Q, Parker-Thornburg J, Capen CC, Cho JY, et al. Targeted expression of the ret/PTC1 oncogene induces papillary thyroid carcinomas. Endocrinology. 1996;137(1):375-8.

23. Santoro M, Chiappetta G, Cerrato A, Salvatore D, Zhang L, Manzo G, et al. Development of thyroid papillary carcinomas secondary to tissue-specific expression of the RET/PTC1 oncogene in transgenic mice. Oncogene. 1996;12(8):1821-6.

24. Powell DJ, Jr., Russell J, Nibu K, Li G, Rhee E, Liao M, et al. The RET/PTC3 oncogene: metastatic solid-type papillary carcinomas in murine thyroids. Cancer Research. 1998;58(23):5523-8.

25. Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, et al. PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell. 1990;60(4):557-63.

26. Santoro M, Dathan NA, Berlingieri MT, Bongarzone I, Paulin C, Grieco M, et al. Molecular characterization of RET/PTC3; a novel rearranged version of the RET proto-oncogene in a human thyroid papillary carcinoma. Oncogene. 1994;9(2):509-16.

27. Pierotti MA, Santoro M, Jenkins RB, Sozzi G, Bongarzone I, Grieco M, et al. Characterization of an inversion on the long arm of chromosome 10 juxtaposing D10S170 and RET and creating the oncogenic sequence RET/PTC. Proceedings of the National Academy of Sciences of the United States of America. 1992;89(5):1616-20.

28. Minoletti F, Butti MG, Coronelli S, Miozzo M, Sozzi G, Pilotti S, et al. The two genes generating RET/PTC3 are localized in chromosomal band 10q11.2. Genes, Chromosomes & Cancer. 1994;11(1):51-7.

29. Klugbauer S, Demidchik EP, Lengfelder E, Rabes HM. Detection of a novel type of RET rearrangement (PTC5) in thyroid carcinomas after Chernobyl and analysis of the involved RET-fused gene RFG5. Cancer Research. 1998;58(2):198-203.

30. Klugbauer S, Jauch A, Lengfelder E, Demidchik E, Rabes HM. A novel type of RET rearrangement (PTC8) in childhood papillary thyroid carcinomas and characterization of the involved gene (RFG8). Cancer Research. 2000;60(24):7028-32.

31. Corvi R, Berger N, Balczon R, Romeo G. RET/PCM-1: a novel fusion gene in papillary thyroid carcinoma. Oncogene. 2000;19(37):4236-42.

32. Saenko V, Rogounovitch T, Shimizu-Yoshida Y, Abrosimov A, Lushnikov E, Roumiantsev P, et al. Novel tumorigenic rearrangement, Delta rfp/ret, in a papillary thyroid carcinoma from externally irradiated patient. Mutation Research. 2003;527(1-2):81-90.

33. Nakata T, Kitamura Y, Shimizu K, Tanaka S, Fujimori M, Yokoyama S, et al. Fusion of a novel gene, ELKS, to RET due to translocation t(10;12)(q11;p13) in a papillary thyroid carcinoma. Genes, Chromosomes & Cancer. 1999;25(2):97-103.

34. Ciampi R, Giordano TJ, Wikenheiser-Brokamp K, Koenig RJ, Nikiforov YE. HOOK3-RET: a novel type of RET/PTC rearrangement in papillary thyroid carcinoma. Endocrine-Related Cancer. 2007;14(2):445-52.

35. Rabes HM, Demidchik EP, Sidorow JD, Lengfelder E, Beimfohr C, Hoelzel D, et al. Pattern of radiation-induced RET and NTRK1 rearrangements in 191 post-chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. Clinical Cancer Research. 2000;6(3):1093-103.

36. Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA. Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Research. 1997;57(9):1690-4.

37. Bounacer A, Wicker R, Caillou B, Cailleux AF, Sarasin A, Schlumberger M, et al. High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumors from patients who had received external

radiation. Oncogene. 1997;15(11):1263-73.

38. Smida J, Salassidis K, Hieber L, Zitzelsberger H, Kellerer AM, Demidchik EP, et al. Distinct frequency of ret rearrangements in papillary thyroid carcinomas of children and adults from Belarus. International Journal of Cancer. 1999;80(1):32-8.

39. Nikiforova MN, Ciampi R, Salvatore G, Santoro M, Gandhi M, Knauf JA, et al. Low prevalence of BRAF mutations in radiation-induced thyroid tumors in contrast to sporadic papillary carcinomas. Cancer Letters. 2004;209(1):1-6.

40. Hamatani K, Eguchi H, Ito R, Mukai M, Takahashi K, Taga M, et al. RET/PTC rearrangements preferentially occurred in papillary thyroid cancer among atomic bomb survivors exposed to high radiation dose. Cancer Research. 2008;68(17):7176-82.

41. Takahashi K, Eguchi H, Arihiro K, Ito R, Koyama K, Soda M, et al. The presence of BRAF point mutation in adult papillary thyroid carcinomas from atomic bomb survivors correlates with radiation dose. Molecular Carcinogenesis. 2007;46(3):242-8.

42. Godbert Y, Henriques de Figueiredo B, Bonichon F, Chibon F, Hostein I, Perot G, et al. Remarkable response to crizotinib in woman with anaplastic lymphoma kinase-rearranged anaplastic thyroid carcinoma. Journal of Clinical Oncology. 2014.

43. Kelly LM, Barila G, Liu P, Evdokimova VN, Trivedi S, Panebianco F, et al. Identification of the transforming STRN-ALK fusion as a potential therapeutic target in the aggressive forms of thyroid cancer. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(11):4233-8.

44. Demeure MJ, Aziz M, Rosenberg R, Gurley SD, Bussey KJ, Carpten JD. Whole-genome sequencing of an aggressive BRAF wild-type papillary thyroid cancer identified EML4-ALK translocation as a therapeutic target. World Journal of Surgery. 2014;38(6):1296-305.

45. Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, et al. PAX8-PPAR gamma1 fusion oncogene in human thyroid carcinoma. Science. 2000;289(5483):1357-60.

46. Gregory Powell J, Wang X, Allard BL, Sahin M, Wang XL, Hay ID, et al. The PAX8/PPAR gamma fusion oncoprotein transforms immortalized human thyrocytes through a mechanism probably involving wild-type PPAR gamma inhibition. Oncogene. 2004;23(20):3634-41.

47. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW, 2nd, Tallini G, et al. RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. The Journal of Clinical Endocrinology and Metabolism. 2003;88(5):2318-26.

48. Dwight T, Thoppe SR, Foukakis T, Lui WO, Wallin G, Hoog A, et al. Involvement of the PAX8/peroxisome proliferator-activated receptor gamma rearrangement in follicular thyroid tumors. The Journal of Clinical Endocrinology and Metabolism. 2003;88(9):4440-5.

49. Marques AR, Espadinha C, Catarino AL, Moniz S, Pereira T, Sobrinho LG, et al. Expression of PAX8-PPAR gamma 1 rearrangements in both follicular thyroid carcinomas and adenomas. The Journal of Clinical Endocrinology and Metabolism. 2002;87(8):3947-52.

50. Castro P, Rebocho AP, Soares RJ, Magalhaes J, Roque L, Trovisco V, et al. PAX8-PPAR gamma rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma. The Journal of Clinical Endocrinology and Metabolism. 2006;91(1):213-20.

51. Landa I, Ganly I, Chan TA, Mitsutake N, Matsuse M, Ibrahimpasic T, et al. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. The Journal of Clinical Endocrinology and Metabolism. 2013;98(9):E1562-6.

52. Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, et al. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocrine-Related Cancer. 2013;20(4):603-10.

53. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339(6122):957-9.

54. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT promoter mutations in familial and sporadic melanoma. Science.

2013;339(6122):959-61.

55. Melo M, da Rocha AG, Vinagre J, Batista R, Peixoto J, Tavares C, et al. TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. The Journal of Clinical Endocrinology and Metabolism. 2014;99(5):E754-65.

56. Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, et al. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. Journal of Clinical Oncology. 2014;32(25):2718-26.

57. Liu X, Qu S, Liu R, Sheng C, Shi X, Zhu G, et al. TERT promoter mutations and their association with BRAF V600E mutation and aggressive clinicopathological characteristics of thyroid cancer. The Journal of Clinical Endocrinology and Metabolism. 2014;99(6):E1130-6.

58. Shi X, Liu R, Qu S, Zhu G, Bishop J, Liu X, et al. Association of TERT promoter mutation 1,295,228 C>T with BRAF V600E mutation, older patient age, and distant metastasis in anaplastic thyroid cancer. The Journal of Clinical Endocrinology and Metabolism. 2015;100(4):E632-7.

59. Jung CK, Little MP, Lubin JH, Brenner AV, Wells SA, Jr., Sigurdson AJ, et al. The increase in thyroid cancer incidence during the last four decades is accompanied by a high frequency of BRAF mutations and a sharp increase in RAS mutations. The Journal of Clinical Endocrinology and Metabolism. 2014;99(2):E276-85.

60. Romei C, Fugazzola L, Puxeddu E, Frasca F, Viola D, Muzza M, et al. Modifications in the papillary thyroid cancer gene profile over the last 15 years. The Journal of Clinical Endocrinology and Metabolism. 2012;97(9):E1758-65.

61. Hong AR, Lim JA, Kim TH, Choi HS, Yoo WS, Min HS, et al. The Frequency and clinical implications of the BRAF(V600E) mutation in papillary thyroid cancer patients in Korea over the past two decades. Endocrinol Metab (Seoul). 2014;29(4):505-13.

62. Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, et al. The association of the BRAF(V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis. Cancer.

2012;118(7):1764-73.
63. Chung KW, Chang MC, Noh DY, Oh SK, Choe KJ, Youn YK. RET oncogene expression of papillary thyroid carcinoma in Korea. Surgery Today. 2004;34(6):485-92.
64. Park KY, Koh JM, Kim YI, Park HJ, Gong G, Hong SJ, et al. Prevalences of Gs alpha, ras, p53 mutations and ret/PTC rearrangement in differentiated thyroid tumours in a Korean population. Clinical Endocrinology. 1998;49(3):317-23.
65. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. The American Journal of Medicine. 1994;97(5):418-28.
66. Alzahrani AS, Mohamed G, Al Shammary A, Aldasouqi S, Abdal Salam S, Shoukri M. Long-term course and predictive factors of elevated serum thyroglobulin and negative diagnostic radioiodine whole body scan in differentiated thyroid cancer. Journal of Endocrinological Investigation. 2005;28(6):540-6.
67. Park do J, Lim JA, Kim TH, Choi HS, Ahn HY, Lee EK, et al. Serum thyroglobulin level measured after thyroxine withdrawal is useful to predict further recurrence in whole body scan-negative papillary thyroid cancer patients after reoperation. Endocrine Journal. 2012;59(11):1021-30.
68. Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, et al. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. Journal of Clinical Oncology. 2015;33(1):42-50.
69. Kelley MJ, Littman SJ. Etiology of the mutational spectrum of ras genes in human carcinomas. Journal of the National Cancer Institute. 2002;94(20):1516-7.
70. Pazzaglia S, Mancuso M, Primerano B, Rebessi S, Biozzi G, Covelli V, et al. Analysis of c-Ha-ras gene mutations in skin tumors induced in carcinogenesis-susceptible and carcinogenesis-resistant mice by different two-stage protocols or tumor promoter alone. Molecular Carcinogenesis. 2001;30(2):111-8.
71. Sills RC, Boorman GA, Neal JE, Hong HL, Devereux TR. Mutations in ras genes in experimental tumours of rodents. IARC Scientific

Publications. 1999(146):55-86.
72. Weihrauch M, Benicke M, Lehnert G, Wittekind C, Wrbitzky R, Tannapfel A. Frequent k- ras -2 mutations and p16(INK4A)methylation in hepatocellular carcinomas in workers exposed to vinyl chloride. British Journal of Cancer. 2001;84(7):982-9.
73. Weihrauch M, Benick M, Lehner G, Wittekind M, Bader M, Wrbitzk R, et al. High prevalence of K-ras-2 mutations in hepatocellular carcinomas in workers exposed to vinyl chloride. International Archives of Occupational and Environmental Health. 2001;74(6):405-10.
74. Porta M, Crous-Bou M, Wark PA, Vineis P, Real FX, Malats N, et al. Cigarette smoking and K-ras mutations in pancreas, lung and colorectal adenocarcinomas: etiopathogenic similarities, differences and paradoxes. Mutation Research. 2009;682(2-3):83-93.
75. Barletta E, Gorini G, Vineis P, Miligi L, Davico L, Mugnai G, et al. Ras gene mutations in patients with acute myeloid leukaemia and exposure to chemical agents. Carcinogenesis. 2004;25(5):749-55.
76. Fukahori M, Yoshida A, Hayashi H, Yoshihara M, Matsukuma S, Sakuma Y, et al. The associations between RAS mutations and clinical characteristics in follicular thyroid tumors: new insights from a single center and a large patient cohort. Thyroid. 2012;22(7):683-9.
77. Jang EK, Song DE, Sim SY, Kwon H, Choi YM, Jeon MJ, et al. NRAS codon 61 mutation is associated with distant metastasis in patients with follicular thyroid carcinoma. Thyroid. 2014;24(8):1275-81.
78. Ohori NP, Nikiforova MN, Schoedel KE, LeBeau SO, Hodak SP, Seethala RR, et al. Contribution of molecular testing to thyroid fine-needle aspiration cytology of "follicular lesion of undetermined significance/atypia of undetermined significance". Cancer Cytopathology. 2010;118(1):17-23.
79. Armstrong MJ, Yang H, Yip L, Ohori NP, McCoy KL, Stang MT, et al. PAX8/PPARgamma rearrangement in thyroid nodules predicts follicular-pattern carcinomas, in particular the encapsulated follicular variant of papillary carcinoma. Thyroid. 2014;24(9):1369-74.
80. Mano H. ALKoma: a cancer subtype with a shared target. Cancer Discovery. 2012;2(6):495-502.

81. Hallberg B, Palmer RH. Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. Nature Reviews Cancer. 2013;13(10):685-700.
82. Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA, Jr., et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(15):6021-6.
83. Liu X, Wu G, Shan Y, Hartmann C, von Deimling A, Xing M. Highly prevalent TERT promoter mutations in bladder cancer and glioblastoma. Cell Cycle. 2013;12(10):1637-8.
84. Gandolfi G, Ragazzi M, Frasoldati A, Piana S, Ciarrocchi A, Sancisi V. TERT promoter mutations are associated with distant metastases in papillary thyroid carcinoma. European Journal of Endocrinology. 2015;172(4):403-13.
85. Cancer Genome Atlas Research N. Integrated genomic characterization of papillary thyroid carcinoma. Cell. 2014;159(3):676-90.

국문초록

한국인 갑상선암의 체성돌연변이의 빈도와 임상상과의 연관성 연구

서울대학교 대학원 의학과 분자유전체의학 전공

임 정 아

서론: 갑상선암의 발생은 최근 40년간 꾸준히 증가하고 있고, 전세계적으로 가장 높은 발생율을 보이고 있다. 초음파를 이용한 갑상선암의 조기진단이 이러한 갑상선암 발생율 증가의 가장 중요한 원인이지만, 다른 환경적인 또는 유전적인 요인도 기여할 것이라고 생각되고 있다. 한국 갑상선유두암의 BRAF 변이의 비율은 70-80%정도로 전 세계에서 가장 높은 비율을 보이고 있다. 반면에 RAS변이나 RET/PTC나 PAX8/PPARγ 재배율과 같은 다른 유전자 변이의 빈도나 임상적 특징에 대한 연구는 아직

미흡하다. 이 연구의 목적은 이 BRAF-V600E 변이의 비율이 높은 지역에서 갑상선암의 체성돌연변이의 비율을 알아보고, 이의 임상적 의의를 찾고자 함이다.

대상과 방법: 서울대학교병원에서 1997년부터 2012년도 까지 갑상선절제술을 받은 갑상선유두암 606명 환자와 갑상선여포암 174명의 환자를 대상으로 후향적 조사를 실시 하였다. RAS와 TERT 변이는 암조직에서 DNA를 추출하여 직접순서결정법 (direct sequencing)을 시행하였고, RET, ALK와 PPARγ 재배열은 암조직의 포르말린 고정 파라핀 내장 조직 (FFPE) 샘플로 형광 제자리 부합법 (FISH)과 면역화학염색을 시행하였다.

결과: BRAF 정상 유두암중 9.2%에서 RAS변이 양성이 나타났고, 각각 NRAS 61 이 18개, NRAS 60이 1개, KRAS 12/13이 2개, HRAS 61이 1개로 전체 유두암에서의 RAS변이 빈도는 2.4%로 추정되었다. RET재배열은 BRAF 정상 유두암에서 8.8%에서 나타났고, 전체 갑상선 유두암에서는 2.6%정도로 추정되었다. RAS 변이는 전형적인 유두암에서보다 여포성변이 유두암에서 더 유의하게 많이 보였다 (29.2% vs 2.8%, $p<0.001$). 전체 유두암에서 전

형적인 유두암은 80.1%였고, 여포성변이 유두암은 14.8%정도를 차지하였다. RAS변이가 있는 PTC가 RAS 정상 종양에 비해 종양 크기가 더 유의하게 컸고 (1.9±1.5cm vs 1.2±0.8cm, p=0.027), 임파선 전이는 더 적게 나타났다 (4.5% vs 40.2%, p=0.001). ALK재배열은 BRAF 정상유두암중 1.8%에서 발견되었으며, 전체 유두암에서는 0.5%정도로 추정되었다. TERT변이는 433개의 유두암중 18개 (4.2%)에서 양성이었고, TERT 음성 유두암에서 재발율이 11.0% (373명중 41명)인 반면 TERT 양성 유두암에서의 재발율은 33.3% (18명중 6명)로 유의하게 더 높았다(hazard ratio [HR], 3.38; 95% CI 1.87 to 8.42; p = 0.005).

여포암에서는 RAS변이가 총 148명중 44명(23.4%)에서 나타났지만, RAS변이암과 RAS정상암에서 임상적 특징의 차이는 없었다. PPARγ 재배열은 61개의 여포암에서 2개에서 양성으로 나타났으나 (3.3%), 유두암에서는 발견되지 않았다. 여포암에서는 TERT 변이 양성율은 5.5%이었고 (109명중 6명 양성), TERT 음성 여포암에서 재발율은 5.5% (109명중 46명)인 반면 TERT 양성 여포암에서의 재발율은 28.6% (7명중 2명)로 유의하게 더 높았다

(HR 7.22; 95% CI 1.39 to 37.45; p = 0.019).

결론: 한국 갑상선 유두암에서 RAS변이의 비율은 낮았고, 이는 여포성 변이 유두암의 비율이 낮았기 때문이었다. RET 재배열의 비율도 낮게 나타나, 한국에서의 갑상선암의 발생원인이 다른나라와 다른 요소가 있을 것으로 생각된다. TERT변이는 유두암 및 여포암에서 불량한 임상상 및 예후와 연관성이 있었다.

주요어: 갑상선유두암, 갑상선여포암, 체성 유전자 변이, 유전자 재배열

학번: 2012-30560