Identification of Innate Drug Resistance against RAF Inhibitors in Patients with Papillary Thyroid Cancer

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Identification of Innate Drug Resistance against RAF Inhibitors in Patients with Papillary Thyroid Cancer

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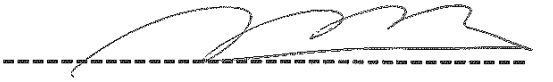

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Proverb 16:9. In his heart a man plans his plans his course, but the LORD determines his steps.

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ABSTRACT

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Recently, several novel targeted agents have been developed for treatment of $BRAF^{V600E}$-positive cancers. However, *de novo* and acquired resistance to these agents has since emerged as a therapeutic obstacle. Two major mechanisms, based on their dependence on RAF dimerization, drive tumor resistance to RAF inhibitors – mutations in NRAS such as NRAS Q61, the $p61BRAF^{V600E}$ splice variant, and C-RAF overexpression are involved in a mechanism that is dependent on RAF dimerization; in contrast, aberrant expression of Cancer Osaka Thyroid Oncogene mitogen-activated protein kinase kinase kinase 8 (COT) or mitogen-activated protein kinase kinase (MEK) mutation function

independently of RAF dimerization. The aim of this study was to identify the molecular basis for innate drug resistance against RAF inhibitors in patients with papillary thyroid cancer (PTC). 167 PTC patients undergoing total thyroidectomy were enrolled. Patient information and clinicopathological parameters were analyzed. $BRAF^{V600E}$ mutation was included in this study. Exome sequencing for detection of mutation in NRAS and MEK was performed. For the analysis of $p61BRAF^{V600E}$ splice variant, DNA sequencing, Western blot and mass spectrometry were performed. CRAF overexpression and aberrant expression of COT were analyzed by quantitative polymerase chain reaction (qPCR) and immunohistochemical staining. In the results, NRAS and MEK mutation, and the $p61BRAF^{V600E}$ splice variant were not detected in PTC. However, qPCR data showed that the relative expression of CRAF and COT mRNA in PTC was higher than in normal tissues ($p<0.01$). Furthermore, COT mRNA expression in PTC correlated positively with CRAF expression ($r=0.5954$, $p<0.001$). Immunohistochemical analysis showed that the staining intensities of COT were higher in PTC than in normal thyroid tissues ($p<0.001$). Aberrant expression of COT was more frequently detected in $BRAF^{V600E}$-positive PTC ($p=0.013$). These results suggest that COT expression may be associated with innate drug resistance against RAF inhibitors in PTC.

Keywords: Papillary thyroid cancer, Drug resistance,Proto-oncogene, BRAF, COT

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I. INTRODUCTION

In recent years, our understanding of the molecular genetics of thyroid cancer has expanded dramatically. Four types of mutations, BRAF and RAS point mutations and RET/PTC and PAX8/peroxisome proliferator-activated receptor-γ rearrangements, constitute the majority of mutations known to occur in the two most common types of thyroid cancer, papillary and follicular carcinoma.

At this time, these genetic changes have the most significant impact on tumor diagnosis and prognostication. Papillary thyroid carcinomas (PTC) harbor point mutations in the BRAF and RAS genes and RET/PTC rearrangements, all of

which promote activation of the mitogen-activated protein kinase (MAPK) pathway. These mutually exclusive mutations are found in >70% of PTC.[1-4]

The RAF family kinases have been shown to play important roles during many cellular and physiological processes, including development, cell cycle regulation, cell proliferation and differentiation, and cell survival and apoptosis. Overexpression or activation of the pathway components is a common indicator in proliferative diseases such as cancer and contributes to tumor initiation, progression, and metastasis.[5]

The RAS-RAF-MAPK pathway is one of the best characterized signal transduction pathways.[6] In this conserved signaling pathway, RAF proteins such as A-, B-, and C-RAF are activated by RAS and then lead to activation of the dual-specific protein kinases mitogen-activated protein kinase kinase (MEK1/2), and subsequently, extracellular signal-regulated kinase (ERK1/2).[6]

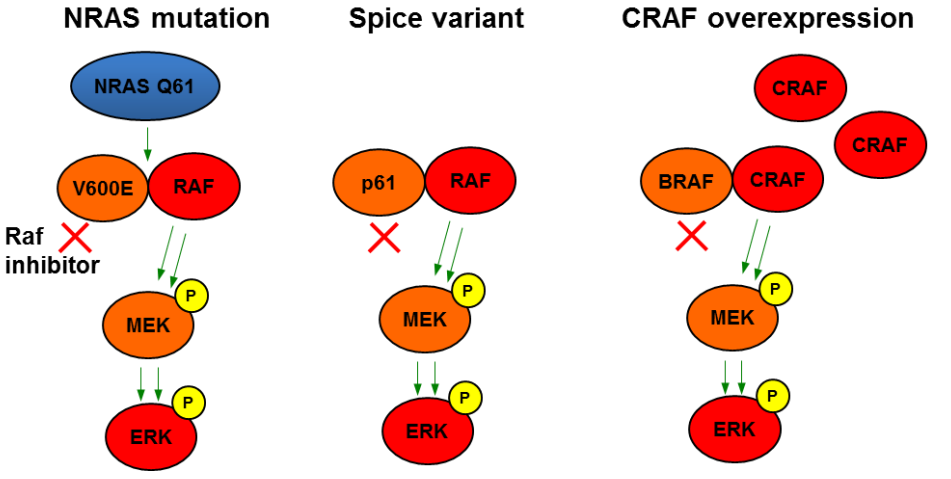
PTC is the result of the abnormal activation of the RAS-RAF-MAPK signal pathway, induced by RET/PTC rearrangement, RAS mutations, or $BRAF^{V600E}$ mutation.[7] Following the discovery that the $BRAF^{V600E}$ mutation is present in a high proportion of many human cancers,[8] several novel targeted agents were developed for $BRAF^{V600E}$-positive cancers.[9] Because the incidence of the $BRAF^{V600E}$ mutation in PTC is high (40%–80%), these new agents were considered promising therapeutic modalities.[10,11]

Preclinical studies indicated the dependency of $BRAFV^{600E}$ tumors on MAPK signaling cascade, whereas the efficacy of both RAF and MEK inhibitors has

been demonstrated in several clinical trials.[12–14] However, de novo and acquired resistance to these agents has since emerged as a new therapeutic obstacle.[15,16]

Mechanisms of resistance to RAF inhibitors can be divided into two categories according to the dependency on RAF dimerization (Fig. 1).[17,18] In the first category, mutations in NRAS such as NRAS Q61, the p61BRAF^{V600E} splice variant, and CRAF overexpression are involved in a mechanism that depends RAF dimerization. The p61BRAF^{V600E} splice variants lacking the RAS-binding domain can dimerize in a RAS-independent manner and generate MEK-ERK signal propagation. NRAS mutation and increased expression of C-RAF can also increase RAF dimerization, which is insensitive to RAF inhibitors (Fig. 1).[19-21] In the second category, aberrant expression of Cancer Osaka Thyroid Oncogene mitogen-activated protein kinase kinase kinase 8 (COT) or MEK mutation (MEK1) functions independently of RAF dimerization.[22] Based on a functional genomic approach, COT can generate resistance to RAF inhibitors by MEK dependent mechanisms.[18,22] The aim of this study was to identify the molecular basis for innate drug resistance against RAF inhibitors in patients with PTC.

(A) RAF dimerization-dependent mechanism



(B) RAF dimerization-independent mechanism

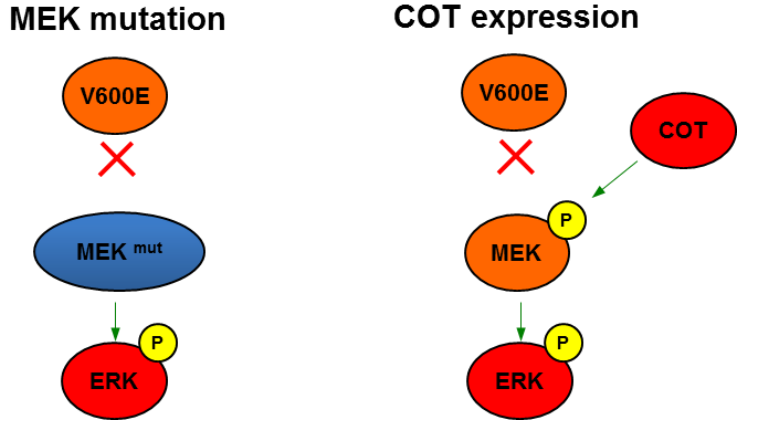


Figure 1. Mechanisms of resistance to RAF inhibitors. (adapted from [18])

(A) RAF dimerization-dependent mechanism

(B) RAF dimerization-independent mechanism

II. MATERIALS AND METHODS

1. Patients and Clinical Manifestation

In this study, 167 patients (34 male and 133 female) undergoing total thyroidectomy were enrolled, with or without neck node dissection followed by radioactive iodine ablation for management of classical PTC, from January 1987 to December 2002 at Severance Hospital, Seoul, South Korea. The study subjects showed no visible remnant in the first Diagnostic 131I whole body scan (WBS), following thyroid hormone withdrawal (THW) performed 6 to 12 months after remnant ablation. The sample size was calculated by Web-based Sample Size/Power Calculations (http://www.stat.ubc.ca). Patient information and clinicopathological parameters were analyzed retrospectively. $BRAF^{V600E}$ mutation was found in 145/167 (86.8%) PTC patients. During this time, recurrence was diagnosed by: histopathologic diagnosis of clinically suspicious lymph node identified by neck ultrasound or physical examination (n = 23, 82.1%); newly detected lesion in 131I diagnostic WBS, 18-Fluoro-deoxyglucose positron emission tomography/computed tomography (FDG PET/CT) or chest CT (n = 5, 17.9%) performed for patients with serum thyroglobulin ≥2 mg/L with gradual increase following THW. Tissue samples were obtained from the central area of the tumor and from contralateral histologically normal tissue. On histological examination, cellularity was >90% in all primary PTCs. All protocols were approved by the institutional review

board of Severance Hospital and written informed consent was obtained from the participants enrolled in the study.

2. Analysis of mutation in NRAS and MEK

Exome sequencing of tissue samples for detection of oncogenic NRAS (Q61K and Q61R) and the MEK1^{C121S} was performed in a central laboratory (Theragen Etex, Suwon, Korea). Tumor samples from 30 patients with PTC were sequenced using an Illumina HiSeq 2000, which produced paired-end, 90-base and 101-base DNA reads. Only tumor cells were collected by macrodissection after hematoxylin staining. To increase the accuracy of mutation detection in genic regions even in low-purity samples, additional exome sequencing was performed at approximately 103 times sequencing depth on average.

3. Analysis of p61BRAF^{V600E} Splice Variant

Genomic DNA from formalin-fixed, paraffin-embedded tissue specimens in 40 patients with PTC was prepared from five 10-mm sections after microdissection. In this case, paraffin-embedded thyroid tissue specimens had >90% tumor cells. Genomic DNA was isolated using the EZ1 DNA Tissue Kit (Qiagen, Chatsworth, CA, USA). Exon 15 of the BRAF gene was amplified by PCR using standard conditions (95℃ for 5 min; followed by 32 cycles of [94℃ for 30 s, 58℃ for 30 s, and 72℃ for 30 s]; and 70℃ for 10 min) using the following primers: forward 5′-ATG CTT GCT CTG ATA GGA AA-3′ and

reverse 5′-ATT TTT GTG AAT ACT GGG GAA-3′. The amplified products were purified with the MinElute PCR Purification Kit (Qiagen) and were then sequenced on an ABI PRISM 3730XL automated capillary DNA Sequencer using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA).Western blotting analysis was performed according to standard methods with commercially available antibodies: B-RAF rabbit polyclonal antibody (sc-9002, Santa Cruz Biotechnology, Inc., Dallas, Texas, USA).

For the Mass spectrometry, Thawed cell samples were first suspended in HEPES buffer (10 mM HEPES, 15 mM MgCl2, 10 mM KCl, and 0.2% DTT) containing protease inhibitors and sonicated. Dissolved proteins were subjected to free-flow electrophoresis (FFE) using a BD™ FFE System (BD Diagnostics, Munich, Germany) with a stable pH gradient between pH 3 (anode) and pH 10 (cathode). After this first separation according to the isoelectric point, fractionated proteins were subjected to SDS-PAGE. Following electrophoresis, the gels were stained with Coomassie Brilliant Blue G-250 (BioRad, Munich, Germany), the lower BRAF bands cut out of the gel and the proteins digested. The proteins of each gel band were analyzed using a nano-LC-MS/MS. The mass spectra obtained were used to identify the corresponding peptides/proteins by the MASCOT™ algorithm. A protein was considered identified when the cumulative score was at least 100.

4. Analysis of CRAF Overexpression and Aberrant Expression of COT

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and complementary DNA (cDNA) was prepared from total RNA using M-MLV reverse transcriptase (Invitrogen) and oligo-dT primers (Promega, Madison, WI, USA). Quantitative RT-PCR (qRT-PCR) was performed on cDNA using the QuantiTect SYBR Green RT-PCR Kit (Qiagen, Valencia, CA, USA) with the following primers: C RAF, 5′-GGG AGC TTG GAA GAC GAT CAG-3′ and 5′-ACA CGG ATA GTG TTG CTT GTC-3′; COT, 5′-ATG GAG TAC ATG AGC ACT GGA-3′ and 5′-GCT GGC TCT TCA CTT GCA TAA AG-3′. The qRT-PCR sample measurements were carried out in triplicate, and three independent trials of each experiment were performed.

Immunohistochemical staining for COT was performed in 167 cases of PTC and matched normal tissues. Briefly, 4-mm tissue sections were heated at 60℃, deparaffinized in xylene, and hydrated in a graded series of alcohol. Antigen retrieval was performed by microwaving in citrate buffer for 10 min. Endogenous peroxidase activity was inactivated by incubating in 3% hydrogen peroxide for 10 min. Nonspecific binding sites were blocked by incubating in 10% normal goat serum diluted with phosphate-buffered saline (PBS). Tissue sections were then incubated with primary antibodies: COT rabbit polyclonal antibody (sc-720) for 60 min at room temperature. All sections were sequentially treated with biotinylated anti-rabbit immunoglobulin for 30 min, peroxidase-labeled streptavidin for 30 min, and diaminobenzidine in the

presence of hydrogen peroxide. Staining was scored as follows: 1, no staining; 2, weak or focal staining; 3, moderate staining in most cells; and 4, strong staining in most cells (Fig.2). To support the data obtained from Immunohistochemistry-Paraffin Embedded Tissue (IHC-P), we reviewed the representative images of IHC-P for COT from the Human Protein Atlas program (http://www.proteinatlas.org/).

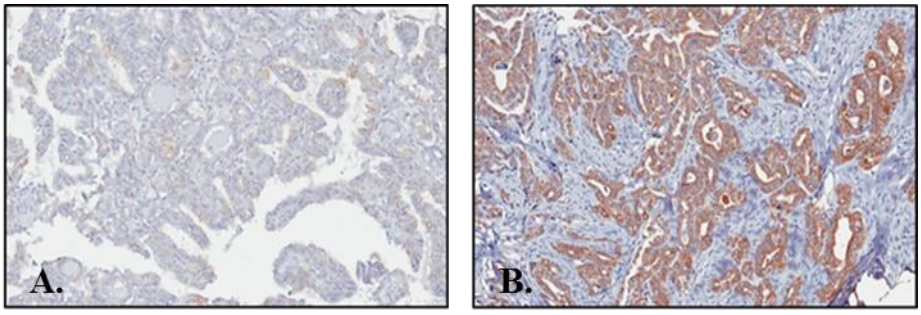


Figure 2. Representative image of immunohistochemical staining of COT in PTC; *(A) No staining, (B) Strong staining*

5. Statistical Analysis

Statistical analysis was carried out using either SPSS version 18.0 for Windows (IBM Corporation, Armonk, NY, USA) or GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). Relative mRNA expression was calculated using the StepOneTM Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Average ratios were compared with the paired t test. The relationship between 2 groups was analyzed by Pearson correlation analysis. Group comparisons were performed by linear-by-linear association. All p values are 2-sided.

III. RESULTS

1. Mutations in NRAS and MEK

Exome sequencing was performed on tumor samples obtained from 30 patients with PTC with an average target depth of 120 per sample. However, oncogenic NRAS (Q61K and Q61R) and the MEK1^{C121S} mutation were not detected in 30 of the PTC patients in this study. (Table. 1)

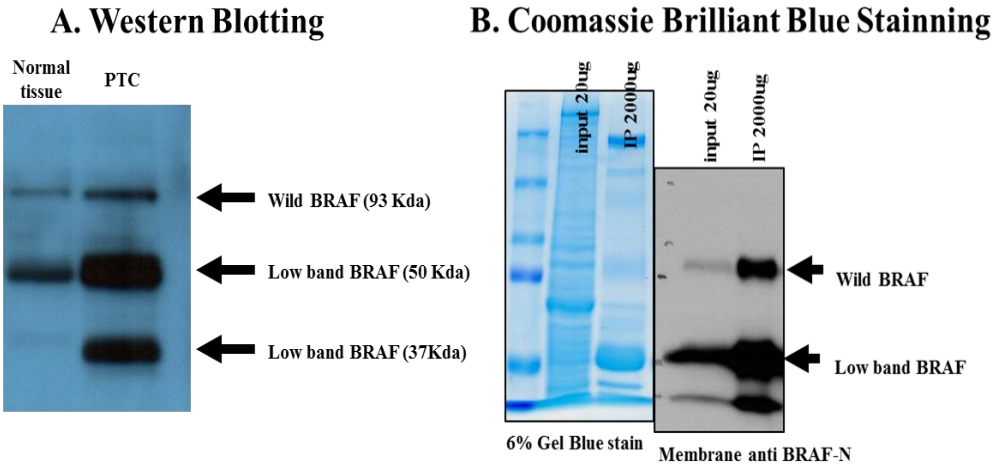
Gene	Samples, n	SNVs in splice site, n	Indels, n
NRAS	30	0	0
MEK	30	0	0

SNVs: single nucleotide variations, Indels: small insertions or deletions

Table 1. Exome sequencing for detection of mutation in NRAS and MEK.

2. p61BRAF^{V600E} splice variant

Analysis of DNA and complementary DNA derived from 40 patients with PTC showed that all retained expression of BRAF^{V600E}. Analysis of BRAF protein expression revealed a 93 kDa band predicted by wild-type braf, and a new band at approximate molecular weights of 50 kDa and 37 kDa was identified in tumor samples from 4 patients. The lower BRAF bands were expected to be splicing variant products of the detected by immunoblotting protein samples, but p61BRAF^{V600E} could not be identify the corresponding proteins by the MASCOT™ algorithm in mass spectrometry (Fig. 3).



C. Mascot Search Results

MATRIX SCIENCE **Mascot Search Results**

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User            : .
Email           : mina@proteometech.com
Search title    :
MS data file    : 150716-YSMC-3 (100).dta
Database        : NCBInr 20150706x (69146588 sequences; 24782014966 residues)
Taxonomy        : Homo sapiens (human) (312165 sequences)
Timestamp       : 20 Jul 2015 at 04:47:48 GMT

Protein hits    : gi|306891    90kDa heat shock protein [Homo sapiens]
                  gi|32488     unnamed protein product [Homo sapiens]
                  gi|62088648  tumor rejection antigen (gp96) 1 variant [Homo sapiens]
                  gi|179832    calnexin [Homo sapiens]
                  gi|1082886   tumor necrosis factor type 1 receptor associated protein TRAP-1 - human
                  gi|181969    elongation factor 2, partial [Homo sapiens]
                  gi|61104919  heat shock protein 90Bf [Homo sapiens]
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Figure 3. Detection of splicing variants of B-RAF; Western Blotting (A), Coomassie Brilliant Blue Stanining (B) and Mascot Search Results (C) *The lower BRAF bands indicate splicing variant products of the detected by immunoblotting protein but, p61BRAF^{V600E} could not be identify the corresponding proteins by the MASCOT™ algorithm in mass spectrometry.*

3. CRAF Overexpression and Aberrant Expression of COT

To investigate the expression of CRAF and COT in PTC, we first performed qPCR using mRNA derived from primary PTC tissues. Excluding 32 PTCs, from which we were only able to isolate mRNA of a quality too poor for further analysis, we conducted mean comparisons to compare the expression of CRAF and COT in PTCs using a paired t-test (n = 135). As shown in Figure 4, the relative mRNA expression of CRAF and COT were higher in PTCs than in normal tissues.

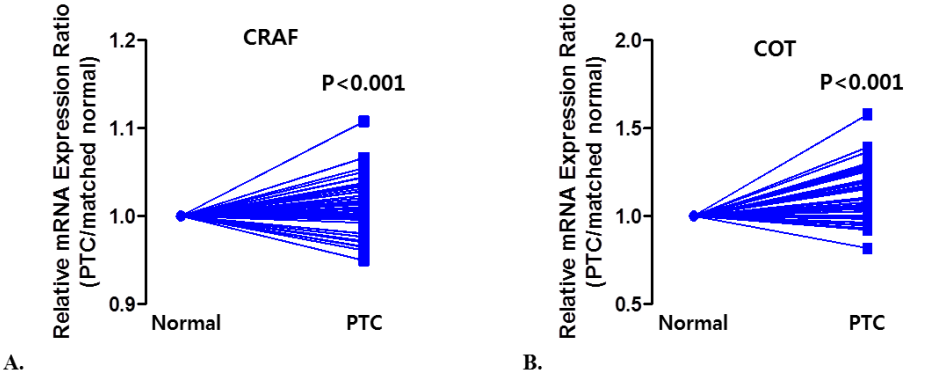

Figure 4. Relative mRNA expression in PTC and matched normal thyroid tissues for CRAF (A), and COT (B) in 135 patients with PTC. *The relative mRNA expression of C-RAF and COT were higher than normal tissues. Average ratios were compared with the paired t test. All p values are two sided. All sample measurements were carried out in triplicate, and three independent trials of each experiment were performed.*

The expression of COT mRNA in PTC showed positive correlation with CRAF (r = 0.5954, p <0.001; Fig. 5)

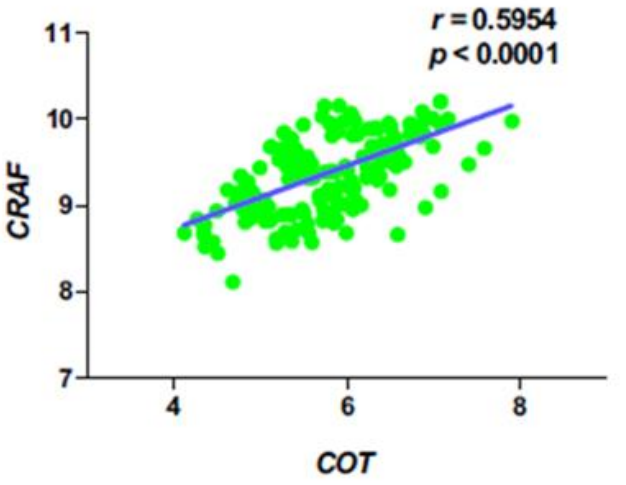

Figure 5. Correlation of COT expression with C-RAF expression in PTC. *The expression of COT mRNA in PTC showed positive correlation with C-RAF (r = 0.5954, p <0.001).* ***The relationship between 2 groups was analyzed by Pearson correlation analysis. r=Pearson correlation coefficient. Statistical analysis was performed using GraphPad Prism.***

PTC tissues showed various staining intensities of COT that ranged from no staining to strong staining and COT expression group comparisons indicated that PTC had significantly higher staining of COT compared with normal thyroid tissues (p<0.001, Fig. 6A). Aberrant expression of COT was more frequently detected in $BRAF^{V600E}$-positive PTC. (p=0.013, Fig. 6B).

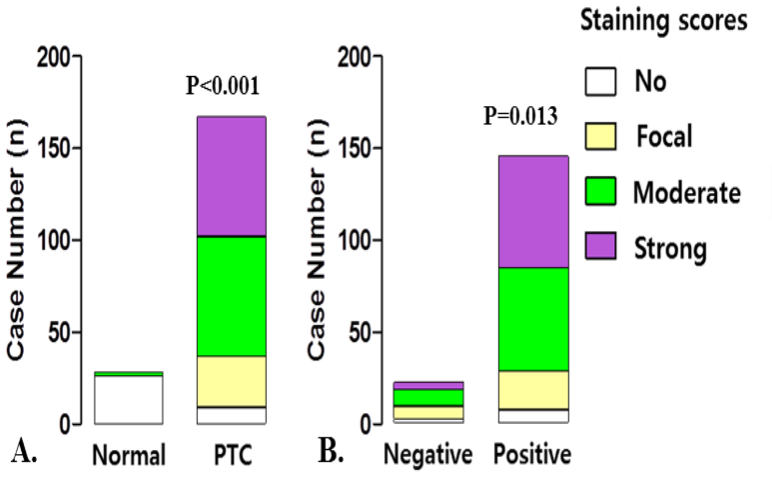

Figure 6. Correlation of COT expression with B-RAFV^{600E} mutation

(A) Comparison of COT expression in normal thyroid tissues and PTC; PTC had significantly higher staining of COT compared with normal thyroid tissues ($p<0.001$). (B) COT expression status according to the absence or presence of B-RAF^{V600E} mutation; Aberrant expression of COT was more frequently detected in BRAF^{V600E}-positive PTC. ($p=0.013$).

IV. DISCUSSION

Following the discovery of the $BRAF^{V600E}$ mutation as an oncogenic kinase in various cancers including melanoma, thyroid, lung, and cholangiocarcinoma, targeted agents against the $BRAF^{V600E}$ kinase have taken a central role in cancer therapy. In this regard, sorafenib has activity against $BRAF^{V600E}$ and is licensed to treat radioactive iodine (RAI)-refractory PTC.[23]

In contrast to the high response rate of metastatic melanomas to BRAF inhibitors, RAF or MEK inhibitors show limited efficacy in RAI-refractory thyroid cancer and thyroid cancer cell lines harboring $BRAF^{V600E}$.[24-26] One of the possible explanations for the poor response to B-RAF inhibitors in thyroid cancer is related to feedback-induced ligand-dependent activation of human epidermal growth factor receptor (HER)2/HER3 signaling.[25] In fact, recent biological and clinical studies have revealed multiple mechanisms of drug resistance: elevated expression of CRAF, COT1, or mutant BRAF kinases; activating mutations in NRAS, MEK1, or AKT1; aberrant splicing of BRAF (p61BRAF); activation of phosphatidylinositol-3-OH kinase by loss of phosphatase and tensin homolog; and activation of receptor tyrosine kinases, including platelet-derived growth factor receptor, beta polypeptide, insulin-like growth factor 1 receptor, and epidermal growth factor receptor. Interactions between tumors and their microenvironment also impact innate drug resistance to BRAF inhibitors.[25]

In the normal physiological setting, activated RAS signaling promotes the dimerization and activation of RAF proteins. In the presence of BRAF inhibitors and RAS signaling, the binding of drug to one molecule in a non-mutated RAF dimer can promote activation of the second RAF molecule. Thus, in the presence of RAS activation, the activity of homo- and hetero-dimeric RAF complexes can be paradoxically activated by RAF inhibitors.[19,27,28] In melanomas with $BRAF^{V600E}$, any alterations promoting RAF dimerization are predicted to confer resistance to RAF inhibitors. As expected, activating NRAS mutations mediate resistance to vemurafenib,[15] a mutated BRAF inhibitor, and dabrafenib,[29] a selective B-RAF kinase inhibitor. Although oncogenic N-RAS (Q61K and Q61R) was not detected in the PTC patients in this study, oncogenic N-RAS (Q61K and Q61R) was only detected in a melanoma patient with acquired resistance to vemurafenib. Also, the alteration frequency of the NRAS gene for thyroid carcinoma sets in the cBioPortal tool (http://www.cbioportal.org/) was only 8.4% (34 of 405 cases).

MEK1 have also been shown to confer resistance to MAPK inhibitors. The existence of MEK1 mutations in MAPK inhibitor-resistant cancers was independently verified in ex vivo cell lines established from tumor material, and its activity in conferring MEK- and BRAF inhibitor resistance validated in transfected melanoma cells. A $MEK1^{C121S}$ mutation was detected via mutational profiling in a melanoma sample from a patient with acquired resistance to vemurafenib.[30] This mutation was not detected in the pre-treatment biopsy, and

was found to confer increased intrinsic kinase activity and resistance to BRAF and MEK inhibition in vitro.[30] The alteration frequency of the MEK1^{C121S} gene for thyroid carcinoma sets in cBioPortal was 0% (0 of 405 cases). Consistent with this, MEK1^{C121S} mutation were not detected in the PTC patients in this study.

Several in-frame BRAF^{V600E} splice variants lacking the RAS-binding domain have been detected in vemurafenib-resistant melanoma tumor specimens.[18] These splice variants contain an in-frame deletion of exons 2–10. These variants lack the RAS-binding domain and can activate ERK in the presence of the inhibitor.[18] In-frame deletion in the p61BRAF^{V600E} variant leads to the constitutive dimerization of BRAF in the absence of activated RAS.[31] Dimerization of p61BRAF^{V600E}, lacking exons 4-8, was critical for mediating B-RAF inhibitor resistance.[21]

In this study, the lower BRAF bands were expected to be splicing variant products of the detected by immunoblotting protein samples, but p61BRAF^{V600E} could not be identify the corresponding proteins by mass spectrometry. It was speculate that BRAF splice variants, as well as NRAF and MEK mutations, may be present, albeit at low levels in BRAF inhibitor treatment-naïve tumors, and, that tumors harboring these alterations likely underwent clonal selection during the course of treatment with a BRAF inhibitor.

RAF proteins such as A-, B-, and CRAF regulate the ERK signaling pathway, and individual RAF isoforms can be differentially regulated in a cell type-specific or context-dependent manner.[32-34] In addition, the RAF isoforms have strikingly different phosphorylation sites.[6] Recently, A-RAF has been reported to act as a scaffold to stabilize BRAF/CRAF heterodimers, whereas ARAF dimerization also promotes MAPK activation.[35,36] Besides the 3 RAF isoforms, COT; (also called MAP3K8), a serine/threonine kinase, was shown to play a role in MAPK activation.[22] To explain the regulatory mechanism of MAPK activation by COT, it has been suggested that COT can phosphorylate MEK1.[37]

In this study, qPCR was performed to estimate the expression of CRAF and COT mRNAs in PTC. Expression of CRAF and COT was increased in PTC. Consistent with the mRNA expression data, western blotting analysis indicated that the expression of CRAF and COT proteins was also increased in PTC. Immunohistochemical analysis clearly demonstrated overexpression or aberrant expression of BRAF and/or COT in PTC, compared with normal thyroid tissues. Furthermore, the expression of COT showed a strong positive correlation with C-RAF expression, suggesting that this de novo drug resistance mechanism is coordinately regulated in PTC. Taken together, we postulated that de novo drug resistance mechanisms to RAF inhibitors might be active in a significant proportion of PTC, coincident with the expression of COT.

Although, mutations in NRAS and MEK, the splice variant p61BRAF^{V600E}

lacking the RAS-binding domain was not detected in the PTC tissues in this study, further genotyping and molecular studies in a larger population of PTC patients, at various stages of treatment, are necessary. De novo drug resistance mechanisms also need to be investigated further so that unnecessary treatment can be avoided.

V. CONCLUSION

CRAF and COT expression levels are higher in PTCs than in normal thyroid tissues. Our data suggest that increased expression of COT might correlate with the innate drug resistance against RAF inhibitors in patients with PTC.

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ABSTRACT(IN KOREAN)

유두상 갑상선암 환자에서 RAF 억제제의 자발적 약제내성 확인

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박 재 현

최근 BRAF 돌연변이 양성 암 치료를 위한 여러 표적 약물이 개발되어 사용되고 있으며 전이성 악성 흑색종 환자에서 무진행 생존기간 연장효과를 확인할 수 있었다. 하지만 유두상 갑상선 암의 경우 RAF 억제제에 대한 효과는 악성 흑색종 환자와 비교해 볼 때 제한적인 것으로 보고되고 있다.

대표적인 RAF 억제제에 대한 약제내성 기전으로는 RAF 이합체화

의존성 기전으로 NRAS 돌연변이, splice 변종 BRAF 돌연변이 (p61BRAF^{V600E} splice variant), CRAF 과발현 등이 있고, RAF 이합체화 독립 기전으로 Cancer Osaka Thyroid Oncogene mitogen-activated protein kinase kinase kinase 8 (COT) 의 비정상적인 발현과 mitogen-activated protein kinase kinase (MEK) 돌연변이 등이 있다. 본 연구에서는 유두상 갑상선 암의 RAF 억제제에 대한 자발적 약제내성 존재를 확인하기 위한 연구를 진행하였다.

본 연구는 유두상 갑상선 암으로 갑상선 전절제 수술을 시행 받은 167명을 대상으로 하였으며 86.8%의 환자에서 BRAF 돌연변이를 동반하였다. NRAS와 MEK에서 돌연변이 검출을 위해 Exome Sequencing 을 시행하였으며, splice 변종 BRAF 돌연변이 분석을 위해 DNA 염기서열 분석 및 Western blot, 질량 분석법 (mass spectrometry) 을 시행하였다. CRAF 과발현과 COT의 비정상적인 발현은 역전사 중합효소 연쇄반응 (reverse transcription polymerase chain reaction, RT-PCR) 과 면역 조직 화학 염색으로 분석 하였다.

본 연구결과 유두상 갑상선암 조직에서 NRAS 돌연변이, MEK 돌연변이, splice 변종 BRAF 돌연변이 등은 검출되지 않았다. 하지만 정상 갑상선 조직과 유두상 갑상선암 조직에서 역전사 중합효소 연쇄반응과 면역 조직 화학 검사를 이용하여 CRAF 와 COT 발현

정도를 비교한 결과 유두상 갑상선 암조직에서 CRAF 와 COT 발현이 증가되었다 (p <0.01). 또한 COT 발현의 발현은 CRAF의 발현과 양의 상관 관계가 있었으며 (p <0.001), 면역 조직 화학 검사결과 정상 갑상선 조직에 비해 유두상 갑상선암 조직에서 COT 염색 강도가 증가되었고 (p <0.01), BRAF 돌연변이 양성 유두상 갑상선암 조직에서 COT의 비정상적인 발현 빈도가 증가하였다 (p =0.013). 이상의 결과를 통하여 유두상 갑상선암 조직에서 COT 발현의 증가는 RAF 억제제에 대한 자발적 약제내성 기전과 관련이 있을 것으로 생각된다.

핵심되는말: 유두상 갑상선암, 약제내성, 원발암유전자, BRAF, COT