

BRAF Mutation from Tissue Samples in Korean Patients with Breast Cancer and Thyroid Cancer: A Pilot Study

Woogyong Kim¹, Hyun Kyung Jung², Yeon Mee Kim¹, Woon Won Kim³, Jung Sun Lee³, Suk Jung Kim²

¹Department of Pathology, Haeundae Paik Hospital, Inje University College of Medicine, Busan, Korea

²Department of Radiology, Haeundae Paik Hospital, Inje University College of Medicine, Busan, Korea

³Department of Surgery, Haeundae Paik Hospital, Inje University College of Medicine, Busan, Korea

Purpose: The purpose of this study was to find the role of BRAF mutation in the development of both breast and thyroid cancers in the same patient in Korean women by evaluating the frequency of the mutation.

Materials and Methods: A total of 16 patients with both breast and thyroid cancers who had undergone surgery from March 2010 to February 2014 were included in this study. We analyzed the frequency of the BRAF V600E mutation in each of the breast and thyroid cancer specimens using dual-priming oligonucleotide based real-time polymerase chain reaction.

Results: The BRAF V600E mutation in patients who had breast and thyroid cancer in Korean women was only identified in thyroid cancer but not in breast cancer and the incidence was 56.3% (9/16).

Conclusion: BRAF V600E mutation, which is the most prevalent mutation in patients of thyroid cancer, does not affect breast cancer in the same patients in Korean population.

Index words: BRAF mutation; Breast cancer; Thyroid cancer; Korean patients

Introduction

Thyroid carcinomas followed by breast carcinomas have become the two leading malignancies in morbidity in women of Korean descent (www.cancer.go.kr, 2012) and several studies have suggested that there is an association in the development of these two disease entities. More than 95% of the thyroid carcinomas are diagnosed as the papillary thyroid carcinoma (PTC) which is the most well differentiated variant and it shows the best prognosis. The risk of second primary malignancies

in women diagnosed with PTC is relatively high compared to other carcinomas because of the young age at diagnosis, early detection due to development of the screening technology, and good long term prognosis (1). Several studies have suggested that there is an increased risk of development of subsequent breast carcinoma in patients with primary (index) thyroid carcinoma (2, 3).

The V600E mutation of the B-type Raf kinase (BRAF) is the most common genetic alteration in thyroid cancer and is detected at a relatively high prevalence (52-86%) in Korean population compared to other countries (15.8-37.5%) (4, 5), especially in conventional PTCs rather than other variants (6). Several studies have reported that the BRAF mutation in PTC plays a key role in the development and progression of the disease, leading to advanced tumor stage, higher risk of

Corresponding to: Hyun Kyung Jung, M.D., Department of Radiology, Haeundae Paik Hospital, Inje University College of Medicine, 875 Haeundae-ro, Haeundae-gu, Busan 612-862, Korea
Tel: 82-51-797-0363 Fax: 82-51-797-0379 E-mail: drsjung@gmail.com

recurrence and poor prognosis (4, 7). However, Davies et al. (8) and Gast et al. (9) demonstrated that the frequency of BRAF mutation is very low in breast cancer and no mutation was present in any of the breast cancers.

Although there is a report about BRAF mutation in melanoma-breast cancer families (9), to our knowledge, no reports on usefulness of BRAF V600E mutation analysis in Korean patients who has both breast and thyroid cancers have been published. Therefore, we retrospectively analyzed the frequency of the BRAF V600E mutation in Korean patients with both breast and thyroid cancers.

Materials and Methods

Patient Selection

The institutional review board approved this retrospective study and informed consents were obtained for this study. Using a computerized search of the database at Inje University Haeundae Paik Hospital from March 2010 to February 2014, 25 patients were identified as those who had undergone surgery for both breast and thyroid cancers. Among them, 7 cases were excluded because informed consents were not obtained and 1 case was excluded due to an unavailable tumor sample of breast carcinoma. All the pathological slides were reviewed by two pathologists blinded to the clinical information. We retrospectively reviewed the surgically confirmed pathologic results and using the patient's medical records, thoroughly collected clinicopathologic data including operation dates, age, side of the tumor location, tumor size, histologic type and grade, lymph node status (LN), estrogen receptor (ER), progesterone receptor (PR) and Human epidermal growth factor receptor-2 (HER2) expressions in both carcinomas. For the presence of any nuclear expression of ER and PR immunohistochemical stains were considered positive (10). HER2 was positive for score 3+ (strong membranous expression of tumor cells >10%) and for any result of an equivocal (score of 2+), silver in situ hybridation was carried out to confirm gene amplification (11).

Tumor Samples

Representative sections of breast and thyroid carcino-

mas from the included patients were selected. A total of 34 tumor specimens were obtained from surgical resections and were fixed in formalin and embedded in paraffin. Tumor samples were sectioned at a thickness of 10 μ m using a sterile microtome blade, resulting in ten tissue samples per case.

DNA Extraction and Analysis of BRAF V600E mutation

Genomic DNA (gDNA) was extracted from ten (10 μ m thickness) slices of formalin-fixed paraffin-embedded (FFPE) material using the QIAamp DNA FFPE extraction kit and the QIAcube automated DNA extraction machine (Qiagen, Hilden, Germany). DNA was quantified by UV absorption (Nanodrop, Thermo Scientific, Wilmington, DE, USA), and typically yielding a total of >1 μ g of gDNA per specimen. We performed all the experimental procedures according to the manufacturer's protocol.

The BRAF mutation was detected using the Anyplex BRAF V600E real time detection system (Seegene Inc., Seoul, Korea). The reaction mixture contained 2 μ L of 10 $\times$ BRAF Oligo Mix (OM) including amplification and detection reagents, 3 μ L of 8-methoxypsoralen (8-Mop) solution to prevent a carryover contamination, 10 μ L of 2 $\times$ Anyplex PCR master mix (Seegene Inc., Seoul, Korea) including DNA polymerase and buffer with deoxynucleoside triphosphates (dNTPs) and mixed. Fifteen μ L of the reaction mixture was dispensed into 0.2-mL polymerase chain reaction (PCR) tubes. The nucleic acid of each sample was added to the reaction mixture tube at a volume of 15 μ L in order to reach a total reaction volume of 20 μ L. The real-time PCR was performed using a CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA) in the following conditions: 15 minutes at 95°C, followed by 15 cycles of 15 seconds at 95°C, 30 seconds at 60°C and then 35 cycles of 30 seconds at 95°C, 32 seconds at 60°C.

For the real-time PCR, the cycle threshold (Ct) was defined as the cycle at which a significant increase in fluorescence is detected. The specimen and the internal control, whose Ct value was < 40, were considered positive controls. Each run contained both a positive and negative control. In addition, if there were any false-negative or false-positive reactions of the control, all the

experimental procedures were repeated again.

Data and Statistical Analysis

The McNemar test was used to compare genetic factors between breast and thyroid tissues. Statistical analyses were performed using SPSS 19.0 for Window software (SPSS, Chicago, IL) and $p < 0.05$ was considered statistically significant.

Results

BRAF mutation result of one thyroid carcinoma was invalid despite three repeated tests due to inadequate specimen preparation. Therefore, a total of 16 patients ($n=16$) were included in this study and retrospectively analyzed for clinical and pathologic data.

Clinicopathologic characteristics of breast and thyroid carcinomas

All patients with both breast and thyroid carcinomas were women. Age at diagnosis of breast and thyroid carcinomas ranged from 32 to 65 years (mean 51.5) and 32 to 64 years (mean 51.1), respectively. The mean time interval between the operations was 184 days, ranging from 0 to 956 days. Four patients were diagnosed simul-

taneously for breast and thyroid cancers through regular health checkups and underwent surgical resections on the same day. Interestingly, the remaining 12 patients had surgical resections for breast cancers prior to secondary thyroid carcinomas which were all revealed through post-operative follow up PET/CT Torso scan. All 16 thyroid carcinomas had histologic type of PTC (100%) and breast cancers were composed of 13 invasive ductal carcinomas not otherwise specified (IDC NOS, 81.3%), 1 tubular carcinoma (TC, 6.3%) and 2 ductal carcinoma in situ (DCIS, 12.5%). The histologic grade of the invasive breast cancers were scored according to the Nottingham histologic score (12) and ranged from 1 to 3 with mean score of 2. The mean size of the PTC was 7.4 mm (range 2–12 mm) and the mean size of the breast cancer was 16.1 mm (range < 1–37 mm). The status of regional nodal involvement was available in 15 of each breast and thyroid cancers and 1 case (6.7%) and 6 cases (50.0%) of breast and thyroid cancers showed nodal metastasis, respectively. The only thyroid cancer case with nodal involvement was included in the 6 cases of breast with nodal metastasis. The overall characteristics of the patients have been summarized in Table 1.

Table 1. Clinicopathologic Characteristics of the 16 Cases in Korean Breast and Thyroid Cancer Patients

Case number	Age	Thyroid				Breast				
		Site	Size (mm)	Lymph node	Pathology	Site	Size (mm)	Lymph node	Pathology	Grade
1	62	Right	8	Positive	PTC	Left	<1	Positive	DCIS	3
2	61	Right	12	Negative	PTC	Left	33	Positive	IDC NOS	2
3	65	Left	5	Negative	PTC	Left	18	Negative	IDC NOS	3
4	61	Left	12	Negative	PTC	Right	5	Positive	IDC NOS	2
5	48	Left	7	Negative	PTC	Right	32	Positive	IDC NOS	1
6	43	Right	2	Not available	PTC	Right	6	Not available	TC	1
7	50	Left	5	Negative	PTC	Right	2	Negative	IDC NOS	1
8	52	Left	6	Negative	PTC	Left	<1	Negative	DCIS	1
9	45	Left	6	Negative	PTC	Right	37	Positive	IDC NOS	2
10	46	Right	10	Negative	PTC	Left	9	Negative	IDC NOS	1
11	62	Left	10	Negative	PTC	Right	19	Negative	IDC NOS	3
12	46	Left	11	Negative	PTC	Left	1	Negative	IDC NOS	1
13	50	Left	5	Negative	PTC	Left	25	Negative	IDC NOS	3
14	43	Left	3	Negative	PTC	Right	13	Negative	IDC NOS	2
15	58	Right	10	Negative	PTC	Left	37	Negative	IDC NOS	3
16	32	Right	6	Negative	PTC	Left	20	Positive	IDC NOS	2

Note.— PTC = papillary thyroid carcinoma, DCIS = ductal carcinoma in situ, IDC NOS = invasive ductal carcinoma not otherwise specified, TC = tubular carcinoma

Molecular profiles of the BRAF mutation and ER, PR, HER2 expressions in breast and thyroid carcinomas

The incidence of BRAF V600E mutation in Korean patients with thyroid cancer was 56.3% (9/16). In contrast, the BRAF V600E mutation was not present in any of the 16 breast cancer tumors. However, apart from 1 case, which could not be tested for ER/PR/Her2 due to inadequate specimen processing, the remaining 15 thyroid tumors showed no ER, PR and HER2 expressions. In breast cancer patients, ER was expressed in 12/16 cases (75%), PR in 11/15 cases (73.3%), and HER2 was present in 3/16 cases (18.8%). Applying McNemar test, comparing BRAF mutation in thyroid and breast cancers of the same patient was statistically analyzed and a significant difference in BRAF mutation between breast and thyroid tissue ($p=0.016$) was identified (Table 2).

We analyzed the correlation between the frequency of the BRAF mutation and other clinicopathologic parameters of the thyroid cancer only as breast cancers were all wild types, and this has been summarized in Table 3. No clinicopathologic variables including age, side of the PTC location, size of the tumor, lymph node metastasis, showed significant correlation with the BRAF mutation in PTC.

Discussion

Although several studies have reported the increased risk of subsequent breast carcinoma in patients with primary thyroid cancer, several studies have suggested that there is no risk of secondary thyroid carcinoma in primary breast cancer patients (2, 3). However, in our study, apart from the 4 cases that were simultaneously diagnosed in regular checkups, the remaining 12 cases

Table 3. Correlation of the BRAF Mutation with Various Clinicopathologic Factors in 16 Patients with Papillary Thyroid carcinoma

Clinicopathologic factors	Number of case	BRAF mutation status	
		Mutant (n=7)	Wild (n=9)
Age(year)			
>55	6	2 (33.3)	4 (66.7)
≤55	10	5 (50.0)	5 (50.0)
Site			
Right	6	4 (66.7)	2 (33.3)
Left	10	3 (30.0)	7 (70.0)
Size(mm)			
≥10	6	4 (66.7)	2 (33.6)
<10	10	3 (30.0)	7 (70.0)
Lymph node metastasis			
Yes	1	0 (0.0)	1 (100.0)
No	14	7 (50.0)	7 (50.0)
Not available	1	0 (0.0)	1 (100.0)

The numbers in parentheses are the percentages.

Table 2. Molecular Profiles from Tissue Samples in Korean Breast and Thyroid Cancer Patients

Case number	Breast				Thyroid			
	BRAF	ER	PR	HER2	BRAF	ER	PR	HER2
1	Negative	Negative	Negative	Negative	Negative	Invalid	Invalid	Invalid
2	Negative	Positive	Positive	Negative	Positive	Negative	Negative	Negative
3	Negative	Positive	Positive	Negative	Positive	Negative	Negative	Negative
4	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative
5	Negative	Positive	Positive	Negative	Positive	Negative	Negative	Negative
6	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Negative
7	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Negative
8	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Negative
9	Negative	Positive	Positive	Negative	Positive	Negative	Negative	Negative
10	Negative	Positive	Positive	Negative	Positive	Negative	Negative	Negative
11	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
12	Negative	Positive	Positive	Positive	Positive	Negative	Negative	Negative
13	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative
14	Negative	Positive	Positive	Negative	Negative	Negative	Negative	Negative
15	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Negative
16	Negative	Positive	Positive	Negative	Positive	Negative	Negative	Negative

Note.— BRAF = B-type Raf kinas mutation, ER = estrogen receptor, PR = progesterone receptor, HER2 = Human epidermal growth factor receptor-2

were diagnosed with breast cancers prior to thyroid tumors, which were revealed in postoperative follow up PET/CT.

Codon 600 is localized in the kinase activation domain and plays a role in over 90% of all mutations identified in the BRAF gene in various cancers. In this study, V600E BRAF mutation study was carried out on 16 patients who had both breast cancer and PTC. 56.3% of PTC cases showed the presence of the BRAF mutation while none of the breast tissue specimens presented the mutations. The frequency of the BRAF mutation in PTC was concordant with the previous reports (52–86%) in Korean population (4, 5). For breast cancer, to our knowledge, the frequency of BRAF mutation in Korean population has not been reported yet, however, V599E BRAF mutation was observed in 2% of breast cancers (8) and no mutant V600E BRAF was found in 39 breast cancer tumors in overseas literature (9). Our result was similar as no mutation was present in any of the breast cancers. There was also no association of the BRAF mutated PTCs with occurrence of breast cancers. As a result of this mutation study, we can suggest that regardless of common genetic susceptibility factors that predispose individuals to breast and thyroid cancer within familiar clusters, the different cancers do not necessarily share somatic genetic events and BRAF mutations remain specific to thyroid in this case.

Estrogens are steroid hormones that control regulation of growth, differentiation and function of the reproductive organs as well as bone, cardiovascular and immune systems in males and females (13). Estrogen and progesterone receptors may be present in breast cancer cells, as their growth depends on estrogen and related hormones, including progesterone. About two-thirds of breast cancers are ER and/or PR positive (<http://www.cancer.net>, 2010). Considering the high morbidity rate and relatively better prognosis of thyroid carcinomas diagnosed in women, especially in premenopausal women, it is suggested that the influence of sex hormones on pathogenesis and development of these thyroid lesions, estrogens in particular, is rather reasonable (14). Estrogen is a potent growth factor both for benign and malignant thyroid cells and plays a growth-stimulating effect via a membrane-bound estrogen receptor which is linked to the MAPK and PI3K ty-

rosine kinase signaling pathways. In PTCs, these pathways may be activated either by a chromosomal rearrangement of the tyrosine receptor kinase TRKA, by RET/PTC genes, or by a BRAF mutation and, in addition, in females they may be stimulated by high levels of estrogen (15). However, ER immunostaining has been variously described, from 0% to over 60% of the thyroid cancer samples in many experimental investigations (16–25).

Simultaneously, ER mRNA detection using reverse transcription PCR ranged from less than 20% to 100% (26, 27). Similarly, for thyroid malignancies, PR expression have been reported in up to 3% to 33% (24, 28) and Her2/neu have been reported up to 80% (29). These discrepancies may be due to technical factors, such as antibodies and also epigenetic modifications of ER/PR expression can play a part. The result of ER, PR and Her2/neu expressions were all negative in PTCs in this study and BRAF mutations in all breast cancers were also negative. Therefore, relationship between hormone receptors, Her2/neu expressions and BRAF mutation in patients with both breast and thyroid cancers could not be determined. To examine this association, a large population-based study is necessary.

The major limitation of this study was that the small number of patients (n=16) were included, which made it difficult to finalize an opinion. Further multicenter studies with a larger group of participants is needed to confirm our study.

In conclusion, our preliminary results showed that the BRAF V600E mutation was not detected in Korean patients with breast cancer and BRAF V600E mutation in PTC did not show any association with the occurrence of breast cancer.

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Conflicts of Interest

No potential conflict of interest relevant to this article

was reported.

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한국 유방암 및 갑상선암 환자 조직에서의 BRAF mutation 여부: 사전 조사

김우경¹ · 정현경² · 김연미¹ · 김운원³ · 이정선³ · 김숙정²

¹인제대학교 의과대학 해운대백병원 병리과

²인제대학교 의과대학 해운대백병원 영상의학과

³인제대학교 의과대학 해운대백병원 외과

목적: 이 연구는 한국 유방암 및 갑상선암으로 진단된 동일한 환자에서 BRAF mutation 빈도를 평가하여 두 질환의 발병에 있어 이의 역할을 알아보고자 한다.

대상 및 방법: 2010년 3월에서 2014년 2월까지 유방암 및 갑상선암으로 수술을 시행한 환자 16명을 대상으로 하였다. 각각의 조직에서 dual-priming oligonucleotide based real-time polymerase chain reaction을 이용하여 BRAF mutation V600E 여부를 분석하였다.

결과: 한국 유방암 및 갑상선암 환자의 조직에서 BRAF V600E mutation은 오직 갑상선암 조직에서만 검출되었고, 유방암 조직에서는 검출되지 않았다. 이의 빈도는 56.3%(9/16)였다.

결론: 갑상선암 환자에서 가장 많은 빈도를 차지하는 BRAF V600E mutation은 같은 환자의 유방암에 영향을 미치지 않는다.

Index words: BRAF mutation; Breast cancer; Thyroid cancer; Korean patients