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Original Article

BRAF mutation in breast cancer by BRAF V600E mutation-specific antibody

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Abstract: The aim of this study is to investigate the BRAF mutation status using BRAF V600E mutation specific antibody in human breast cancer tissue, and discuss its clinical implications. Immunohistochemical staining for BRAF V600E mutation specific antibody was performed using tissue microarrays of 230 cases of breast cancer and 132 cases of triple-negative breast cancer (TNBC). The cases were subdivided into four molecular subtypes, luminal A, luminal B, HER-2 or TNBC, according to the results of ER, PR, HER-2, Ki-67 immunohistochemistry and HER-2 FISH. In TNBC cases, additional immunohistochemical stain for CK5/6, EGFR, claudin 3, claudin 4, claudin 7, E-cadherin, AR, GGT-1, STAT1, and interleukin-8 were performed. TNBC cases were then further subcategorized as follows: *basal-like type (CK5/6* positive *and/or EGFR* positive*), molecular apocrine type (AR* positive *and/or GGT-1* positive*), claudin low type* (claudin 3, claudin 4, claudin 7 negative *and* E-cadherin negative*), immune related type (stromal STAT1 positive and IL-8 negative), mixed type (*cases consisting of two or more mixed components), and *null type* (cases that cannot be categorized in any of abovementioned types). In 230 breast cancer, 30 (13.0%) cases showed positivity for BRAF V600E mutation specific antibody, and 17 (7.4%) showed nuclear expression. The nuclear BRAF V600E positivity was associated with ER negativity (P=0.003), PR negativity (P=0.031) and TNBC subtype (P=0.009). In 132 cases of TNBC, 4 (3.0%) cases were positive for BRAF V600E mutation specific antibody, and 10 (7.6%) cases showed nuclear expression. BRAF V600E positivity was most frequently found in the null type, followed by mixed type and basal-like type, and was not found in other subtypes. In TNBC, the nuclear BRAF V600E positivity was associated with lower histological grade (P=0.012). BRAF V600E status did not correlate with the prognosis of breast cancers and TNBC. In conclusion, positivity for BRAF V600E mutation specific antibody was noted in a fraction of breast cancer and TNBC, suggesting the presence of BRAF mutation. BRAF mutation did not have association with clinicopathologic factors of breast cancer.

Keywords: Breast cancer, BRAF mutation, immunohistochemistry

Introduction

BRAF is a member of Raf kinase family proteins, weighing 75-100 kDa, and it is the most important activator of MEK kinase in Ras-Raf-MEK-ERK pathway [1, 2]. In certain tumors, Ras-Raf-MEK-ERK pathway is abnormally activated; the BRAF mutation is a typical cause of aberrant ERK signaling [3]. BRAF mutation was first reported in 2002, and in 90% of them, a missense mutation occurs at nucleotide 1796, and this results in valine to glutamic acid substitution at codon 599 (V599E; later renamed to V600E due to nomenclature change [4]. BRAF V600E mutation is reported to be found in various neoplasms, and the reported prevalence in tumors are as follows; malignant melanoma (40-70%), colorectal carcinoma (5-22%), thyroid papillary carcinoma (36-53%), glioma (11%), ovary serous carcinoma (30%), lung adenocarcinoma (4%) and hairy cell leukemia (100%) [5]. The gold standard method for detecting BRAF mutation is the Sanger sequencing method, but the test is expensive and it requires expensive equipment. To overcome these drawbacks, immunohistochemistry (IHC) method using BRAF V600E mutation specific antibody was introduced [6]. In a study performed with MSI-H colorectal carcinomas, this method showed a high concordance rate (98.9%) with Sanger sequencing method, and 100% of sensitivity and 98.8% of specificity [7], revealing IHC by BRAF V600E mutation specific antibody is an effective surrogate method for evaluating BRAF V600E mutation.

Table 1. Source, clone, and dilution of used antibodies

Antibody	Clone	Dilution	Company
BRAF V600E mutation related			
BRAF V600E	VE1	1:50	Spring Bioscience, Pleasanton, CA, USA
Molecular subtype related			
ER	SP1	1:100	Thermo Scientific, San Diego, CA, USA
PR	PgR	1:50	DAKO, Glostrup, Denmark
HER-2	Polyclonal	1:1500	DAKO, Glostrup, Denmark
Ki-67	MIB-1	1:150	DAKO, Glostrup, Denmark
TNBC molecular subtype related			
CK5/6	D5/16B4	1:50	DAKO, Glostrup, Denmark
EGFR	EGFR.25	1:50	Novocastra, Newcastle, UK
AR	AR441	1:50	DAKO, Glostrup, Denmark
GGT-1	IgG2A	1:50	Abcam, Cambridge, UK
Claudin 3	Polyclonal	1:50	Abcam, Cambridge, UK
Claudin 4	Polyclonal	1:100	Abcam, Cambridge, UK
Claudin 7	Polyclonal	1:100	Abcam, Cambridge, UK
E-cadherin	36B5	1:100	Novocastra, Newcastle, UK
STAT1	Polyclonal	1:100	Abcam, Cambridge, UK
Interleukin-8	807	1:50	Abcam, Cambridge, UK

Breast cancer is a neoplasm that is well known to have genetic aberration and genetic heterogeneity [8]. In previous studies, in about 10% of breast cancer cell lines had BRAF mutation [9], suggesting the possible presence of BRAF mutation in breast cancer tissues. A few studies on BRAF mutations in breast cancer tissues have been performed so far, using sequencing methods [10, 11], but studies that used BRAF V600E mutation specific antibody are rare. The aim of this study is to investigate the BRAF mutation status using IHC with BRAF V600E mutation specific antibody, and its clinical implications.

Materials and methods

Patient selection and histologic evaluation

In this study, two groups of breast cancers are involved. The first group involves the patients who were diagnosed with invasive ductal carcinoma (IDC), NOS from January, 2006 to December, 2006 at Severance hospital. In the second group, patients who were diagnosed with triple negative breast cancer (TNBC), from January 2000 to December 2005 at Severance hospital were enrolled. Patients who had preoperative chemotherapy or hormone therapy were excluded. This study was approved by the Institutional Review Board of Yonsei University Severance Hospital. IRB exempted the informed consent from patients. All cases were reviewed by a breast pathologist (Koo JS) with Hematoxylin & Eosin (H&E)-stained slides. Histological grade was assessed using the Nottingham grading system [12]. Clinicopathologic parameters evaluated in each case included patient age at initial diagnosis, lymph node metastasis, tumor recurrence, and patient survival.

The tumor stroma of IDC were subcategorized into four types as following; 1) desmoplastic type: in cases the tumor stroma consists of cellular fibroblast/myofibroblast proliferation, 2) sclerotic type: in cases fibrotic collagenous component predominates with little cancer cell component, 3) pauci type; in cases with no stromal reaction or in cases with normal breast stroma, 4) inflammatory type: in cases when the inflammatory cells such as lymphocyte predominates the tumor stroma.

In TNBC, following histologic features were evaluated; apocrine histology, central fibrotic zone, and lymphocyte infiltration. The apocrine histology was defined by abundant granular eosinophilic cytoplasm, cytoplasmic vacuolization, and vesicular nuclei with prominent nucleoli in more than 10% of tumor cells.

Table 2. Clinicopathologic characteristics of patients according to breast cancer molecular subtypes

Parameter	Total (n=230) (%)	Luminal A (n=115) (%)	Luminal B (n=57) (%)	HER-2 (n=15) (%)	TNBC (n=43) (%)	*P*-value
Age (years)						0.087
≤50	131 (57.0)	66 (57.4)	39 (68.4)	7 (46.7)	19 (44.2)	
>50	99 (43.0)	49 (42.6)	18 (31.6)	8 (53.3)	24 (55.8)	
Histologic grade						<0.001
I/II	163 (70.9)	105 (91.3)	36 (63.2)	8 (53.3)	14 (32.6)	
III	67 (29.1)	10 (8.7)	21 (36.8)	7 (46.7)	29 (67.4)	
Tumor stage						0.112
T1	146 (63.5)	80 (69.6)	35 (61.4)	10 (66.7)	21 (48.8)	
T2/T3	84 (36.5)	35 (30.4)	22 (38.6)	5 (33.3)	22 (51.2)	
Nodal metastasis						0.555
Absent	147 (63.9)	69 (60.0)	37 (64.9)	10 (66.7)	31 (72.1)	
Present	83 (36.1)	46 (40.0)	20 (35.1)	5 (33.3)	12 (27.9)	
Estrogen receptor status						<0.001
Negative	63 (27.4)	2 (1.7)	3 (5.3)	15 (100.0)	43 (100.0)	
Positive	167 (72.6)	113 (98.3)	54 (94.7)	0 (0.0)	0 (0.0)	
Progesterone receptor status						<0.001
Negative	80 (34.8)	10 (8.7)	12 (21.1)	15 (100.0)	43 (100.0)	
Positive	150 (65.2)	105 (91.3)	45 (78.9)	0 (0.0)	0 (0.0)	
HER-2 status						<0.001
Negative	185 (80.4)	115 (100.0)	27 (47.4)	0 (0.0)	43 (100.0)	
Positive	45 (19.6)	0 (0.0)	30 (52.6)	15 (100.0)	0 (0.0)	
Ki-67 LI (%)						<0.001
≤14	144 (62.6)	115 (100.0)	18 (31.6)	6 (40.0)	5 (11.6)	
>14	86 (37.4)	0 (0.0)	39 (68.4)	9 (60.0)	38 (88.4)	
Stromal type						0.007
Desmoplastic	86 (37.4)	40 (34.8)	28 (49.1)	6 (40.0)	12 (27.9)	
Lymphocytic	16 (7.0)	3 (2.6)	3 (5.3)	1 (6.7)	9 (20.9)	
Pauci	9 (3.9)	4 (3.5)	3 (5.3)	0 (0.0)	2 (4.7)	
Sclerotic	119 (51.7)	68 (59.1)	23 (40.4)	8 (53.3)	20 (46.5)	
Tumor recurrence	11 (4.8)	4 (3.5)	2 (3.5)	1 (6.7)	4 (9.3)	0.444
No. of patient deaths	18 (7.8)	6 (5.2)	3 (5.3)	2 (13.3)	7 (16.3)	0.090

Note: TNBC, triple negative breast cancer.

Tissue microarray

A representative area showing tumor and tumor stroma was selected on an H&E-stained slide, and a corresponding spot was marked on the surface of the paraffin block. Using a biopsy needle, the selected area was punched out, and a 3-mm tissue core was transferred to a 6 × 5 recipient block. Two tissue cores of invasive tumor were extracted to minimize extraction bias. Each tissue core was assigned a unique tissue microarray location number that was linked to a database containing other clinicopathologic data.

Immunohistochemistry

Antibodies used for IHC are listed in **Table 1**. All IHC was performed with formalin-fixed, paraffin-embedded tissue sections using an automatic IHC staining device (Benchmark XT, Ventana Medical System, Tucson, AZ, USA). Briefly, 5-μm-thick formaldehyde fixed paraffin-embedded tissue sections were transferred onto adhesive slides and dried at 62°C for 30 minutes. Standard heat epitope retrieval was performed for 30 minutes in ethylene diamine tetraacetic acid, pH 8.0, in the autostainer. The samples were then incubated with primary anti-

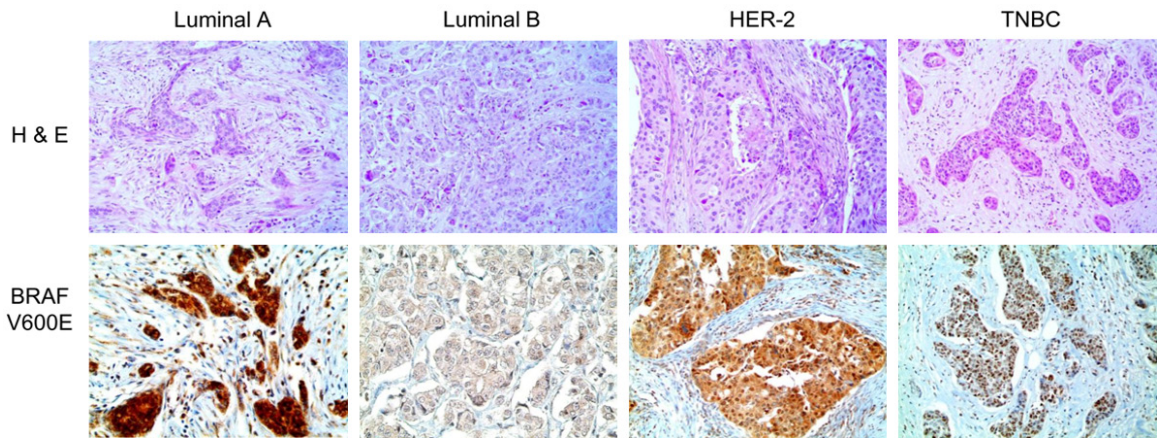

Figure 1. Expression of BRAF V600E mutation specific antibody in immunohistochemistry according to the molecular subtype in breast cancer. In luminal A and HER 2 type, homogenous and diffuse cytoplasmic expression is noted, while triple negative breast cancer shows nuclear expression.

bodies. After incubation with primary antibodies, The sections were subsequently incubated with biotinylated anti-mouse immunoglobulins, peroxidase-labeled streptavidin (LSAB kit, DakoCytomation), and 3,30-diaminobenzidine. Negative control samples were processed without the primary antibody. Slides were counterstained with Harris hematoxylin. Positive control tissue was used as per the manufacturer's recommendation. Slides were counterstained with Harris hematoxylin.

Interpretation of immunohistochemical staining

All immunohistochemical markers were accessed by light microscopy. A cut-off value of 1% or more positively stained nuclei was used to define ER and PR positivity [13]. HER-2 staining was analyzed according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines using the following categories: 0 = no immunostaining; 1+ = weak incomplete membranous staining, less than 10% of tumor cells; 2+ = complete membranous staining, either uniform or weak in at least 10% of tumor cells; and 3+ = uniform intense membranous staining in at least 30% of tumor cells [14]. HER-2 immunostaining was considered positive when strong (3+) membranous staining was observed, whereas cases with 0 to 1+ were regarded as negative. Cases showing 2+ HER-2 expression were evaluated for HER-2 amplification by fluorescent *in situ* hybridization (FISH). Ki-67 labeling index (LI) was defined as the percentage of nuclear-positive cells among the tumor cells.

Immunohistochemical markers for TNBC molecular subtype were accessed by light microscopy. The stained slides were evaluated semi quantitatively [15]. Tumor and stromal cell staining were assessed as 0: negative or weak immunostaining in <1% of the tumor/stroma, 1: focal expression in 1-10% of tumor/stroma, 2: positive in 11%-50% of tumor/stroma, and 3: positive in 51%-100% of tumor/stroma. The evaluation was performed throughout the whole area of the tumor, and score 0 was regarded negative, and 1 or more was recorded positive. Cases with 20% or more positive tumor cells were recorded BRAF V600E positive [16].

Tumor phenotype classification

In this study, we classified breast cancer phenotypes according to the IHC results for ER, PR, HER-2, Ki-67 and FISH results for HER-2 as follows [17]: *luminal A type*, ER or/and PR positive, HER-2 negative and Ki-67 LI <14%; *Luminal B type*, (HER-2 negative) ER or/and PR positive, HER-2 negative and Ki-67 LI ≥14%; (HER-2 positive) ER or/and PR positive and HER-2 overexpressed or/and amplified; *HER-2 overexpression type*, ER and PR negative and HER-2 overexpressed or/and amplified; *TNBC type*: ER, PR, and HER-2 negative.

According to the results of IHCs, TNBC were sub-classified as below [15]; *basal-like type*

Table 3. Clinicopathologic characteristics of patients according to the status of BRAF V600E immunohistochemistry

Parameter	BRAF V600E			Nuclear BRAF V600E		
	Negative n=200 (%)	Positive n=30 (%)	*P*-value	Negative n=213 (%)	Positive n=17 (%)	*P*-value
Age (years)			0.409			0.872
≤50	116 (58.0)	15 (50.0)		121 (56.8)	10 (58.8)	
>50	84 (42.0)	15 (50.0)		92 (43.2)	7 (41.2)	
Histologic grade			0.238			0.979
I/II	139 (69.5)	24 (80.0)		151 (70.9)	12 (70.6)	
III	61 (30.5)	6 (20.0)		62 (29.1)	5 (29.4)	
Tumor stage			0.426			0.348
T1	125 (62.5)	21 (70.0)		137 (64.3)	9 (52.9)	
T2/T3	75 (37.5)	9 (30.0)		76 (35.7)	8 (47.1)	
Nodal metastasis			0.632			0.263
Absent	129 (64.5)	18 (60.0)		134 (62.9)	13 (76.5)	
Present	71 (35.5)	12 (40.0)		79 (37.1)	4 (23.5)	
Estrogen receptor status			0.593			0.003
Negative	56 (28.0)	7 (23.3)		53 (24.9)	10 (58.8)	
Positive	144 (72.0)	23 (76.7)		160 (75.1)	7 (41.2)	
Progesterone receptor status			0.858			0.031
Negative	70 (35.0)	10 (33.3)		70 (32.9)	10 (58.8)	
Positive	130 (65.0)	20 (66.7)		143 (67.1)	7 (41.2)	
HER-2 status			0.949			0.669
Negative	161 (80.5)	24 (80.0)		172 (80.8)	13 (76.5)	
Positive	39 (19.5)	6 (20.0)		41 (19.2)	4 (23.5)	
Ki-67 LI (%)			0.751			0.169
≤14	126 (63.0)	18 (60.0)		136 (63.8)	8 (47.1)	
>14	74 (37.0)	12 (40.0)		77 (36.2)	9 (52.9)	
Stromal type			0.705			0.412
Desmoplastic	74 (37.0)	12 (40.0)		82 (38.5)	4 (23.5)	
Lymphocytic	15 (7.5)	1 (3.3)		14 (6.6)	2 (11.8)	
Pauci	7 (3.5)	2 (6.7)		9 (4.2)	0 (0.0)	
Sclerotic	104 (52.0)	15 (50.0)		108 (50.7)	11 (64.7)	
Molecular subtype			0.266			0.009
Luminal A	99 (49.5)	16 (53.3)		110 (51.6)	5 (29.4)	
Luminal B	50 (25.0)	7 (23.3)		55 (25.8)	2 (11.8)	
HER-2	11 (5.5)	4 (13.3)		13 (6.1)	2 (11.8)	
TNBC	40 (20.0)	3 (10.0)		35 (16.4)	8 (47.1)	

Note: TNBC, triple negative breast cancer.

(CK5/6 positive *and/or EGFR* positive*), molecular apocrine type (AR* positive *and/or GGT-1* positive*), claudin low type* (claudin 3, claudin 4, claudin 7 negative *and* E-cadherin negative*), immune related type (stromal STAT1 positive and IL-8 negative), mixed type (*cases consisting of two or more mixed components), and *null type* (cases that cannot be categorized in any of abovementioned types).

Statistical analysis

Data were analyzed using SPSS for Windows, Version 12.0 (SPSS Inc., Chicago, IL, USA). For determination of statistical significance, Student's *t* and Fisher's exact tests were used for continuous and categorical variables, respectively. In the case of analyzing data with multiple comparisons, a corrected *p*-value with

Table 4. Clinicopathologic characteristics of patients according to triple negative breast cancer phenotype

Parameters	*Total (n=132) (%)*	*Basal-like type (n=55) (%)*	*Molecular apocrine type (n=11) (%)*	*Claudine low type (n=8) (%)*	*Immune related type (n=6) (%)*	*Mixed type (n=29) (%)*	*Null type (n=23) (%)*	*P-value*
Age (years)								0.784
≤50	77 (58.3)	36 (65.5)	5 (45.5)	4 (50.0)	3 (50.0)	16 (55.2)	13 (56.5)	
>50	55 (41.7)	19 (34.5)	6 (54.5)	4 (50.0)	3 (50.0)	13 (44.8)	10 (43.5)	
Histologic grade								0.586
I/II	42 (31.8)	17 (30.9)	6 (54.5)	3 (37.5)	1 (16.7)	9 (31.0)	9 (26.1)	
III	90 (68.2)	38 (69.1)	5 (45.5)	5 (62.5)	5 (83.3)	20 (69.0)	17 (73.9)	
Tumor stage								0.719
T1	50 (37.9)	24 (43.6)	5 (45.5)	3 (37.5)	1 (16.7)	10 (34.5)	7 (30.4)	
T2/T3	82 (62.1)	31 (56.4)	6 (54.5)	5 (62.5)	5 (83.3)	19 (65.5)	16 (69.6)	
Nodal metastasis								0.459
No	86 (65.2)	40 (72.7)	6 (54.5)	5 (62.5)	5 (83.3)	18 (62.1)	12 (52.2)	
Yes	46 (34.8)	15 (27.3)	5 (45.5)	3 (37.5)	1 (16.7)	11 (37.9)	11 (47.8)	
Central acellular zone								0.224
No	101 (76.5)	38 (69.1)	11 (100.0)	6 (75.0)	6 (100.0)	22 (75.9)	18 (78.3)	
Yes	31 (23.5)	17 (30.9)	0 (0.0)	2 (25.0)	0 (0.0)	7 (24.1)	5 (21.7)	
Central necrotic zone								0.877
No	123 (93.2)	51 (92.7)	11 (100.0)	7 (87.5)	6 (100.0)	27 (93.1)	21 (91.3)	
Yes	9 (6.8)	4 (7.3)	0 (0.0)	1 (12.5)	0 (0.0)	2 (6.9)	2 (8.7)	
Central fibrotic zone								0.351
No	106 (80.3)	41 (74.5)	11 (100.0)	6 (75.0)	6 (100.0)	23 (79.3)	19 (82.6)	
Yes	26 (19.7)	14 (25.5)	0 (0.0)	2 (25.0)	0 (0.0)	6 (20.7)	4 (17.4)	
Lymphocytic infiltration								0.025
Absent	99 (75.0)	41 (74.5)	8 (72.7)	7 (87.5)	2 (33.3)	19 (65.5)	22 (95.7)	
Present	33 (25.0)	14 (25.5)	3 (27.3)	1 (12.5)	4 (66.7)	10 (34.5)	1 (4.3)	
Tumor cell discohesiveness								0.082
No	122 (92.4)	53 (96.4)	8 (72.7)	7 (87.5)	6 (100.0)	28 (96.6)	20 (87.0)	
Yes	10 (7.6)	2 (3.6)	3 (27.3)	1 (12.5)	0 (0.0)	1 (3.4)	3 (13.0)	
Tumor margin								0.165
Expanding	112 (84.8)	49 (89.1)	8 (72.7)	7 (87.5)	6 (100.0)	26 (89.7)	16 (69.6)	
Infiltrative	20 (15.2)	6 (10.9)	3 (27.3)	1 (12.5)	0 (0.0)	3 (10.3)	7 (30.4)	
Apocrine differentiation								0.026
No	108 (81.8)	47 (85.5)	5 (45.5)	7 (87.5)	4 (66.7)	24 (82.8)	21 (91.3)	
Yes	24 (18.2)	8 (14.5)	6 (54.5)	1 (12.5)	2 (33.3)	5 (17.2)	2 (8.7)	
Ki 67 LI (%, mean ± SD)	27.3±23.3	33.3±24.6	6.0±4.8	27.3±27.8	38.6±27.5	28.5±22.4	20.7±17.4	0.005
Tumor recurrence	14 (10.6)	7 (12.7)	1 (9.1)	1 (12.5)	0 (0.0)	1 (3.4)	4 (17.4)	0.591
Patient death	14 (10.6)	7 (12.7)	1 (9.1)	1 (12.5)	1 (16.7)	0 (0.0)	4 (17.4)	0.403

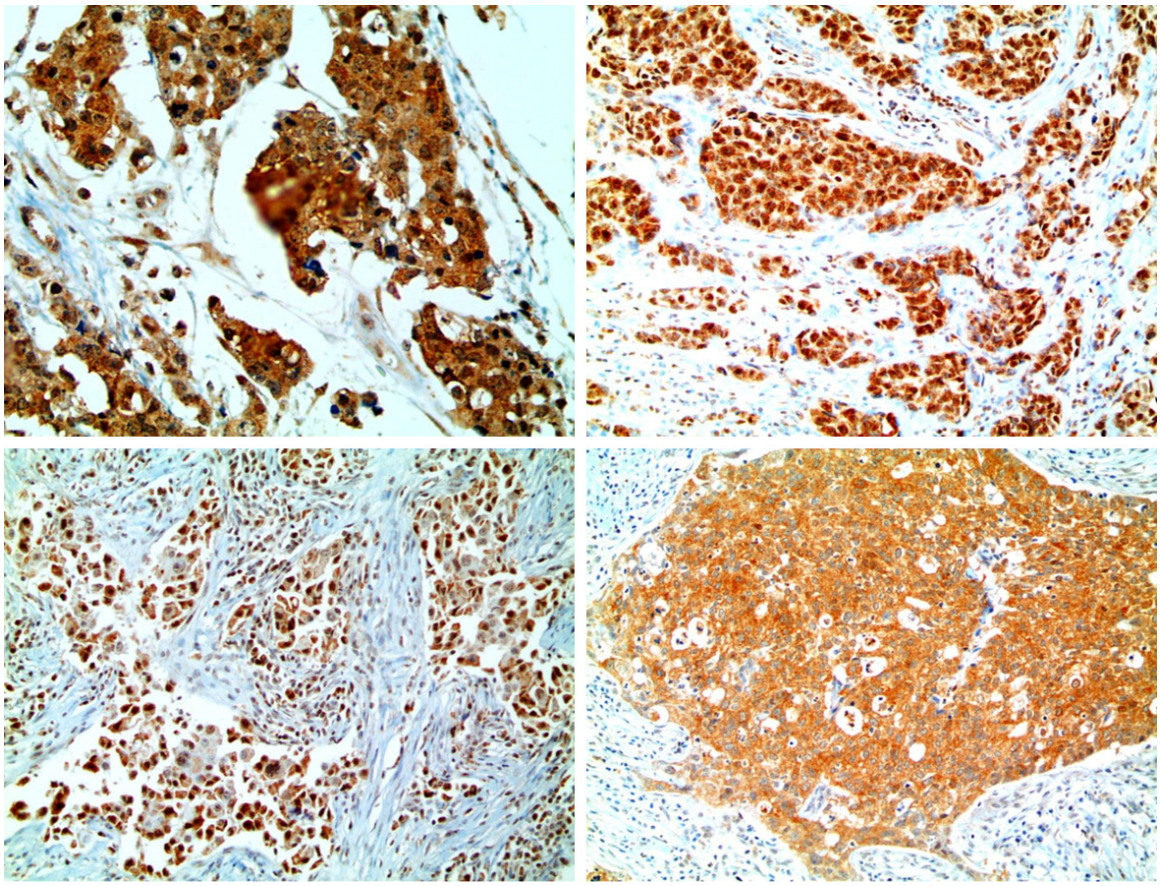

Figure 2. Representative positive cases for BRAF V600E mutation specific antibody in triple negative breast cancer.

the application of the Bonferroni multiple comparison procedure was used. Statistical significance was set to $P<0.05$. Kaplan-Meier survival curves and log-rank statistics were employed to evaluate time to tumor recurrence and overall survival. Multivariate regression analysis was performed using the Cox proportional hazards model.

Results

Basal characteristics of breast cancer

In 230 breast cancers, 115 (50.0%), 57 (24.8%), 15 (6.5%), and 43 (18.7%) were of luminal A, luminal B, HER-2 and TNBC subtype, respectively. TNBC subtype had higher histological grade ($P<0.001$), higher Ki-67 LI ($P<0.001$) compared to other subtypes. The stromal types were different according to molecular subtypes ($P=0.007$), with higher proportion of lymphocytic type in TNBC subtype, higher rate of desmoplastic type in luminal B subtype and higher rate of sclerotic type in other molecular subtypes (**Table 2**).

Expression of BRAF V600E mutation specific antibody in breast cancer

The immunohistochemical expression of BRAF V600E mutation specific antibody was investigated in breast cancers. Thirty (13.0%) cases showed cytoplasmic expression, 17 (7.4%) showed nuclear expression (**Figure 1**). On an analysis of correlation between the clinicopathologic parameters and BRAF V600E status, cytoplasmic BRAF V600E status did not show an association with clinicopathologic parameters. On the other hand, nuclear BRAF V600E status was associated with ER negativity ($P=0.003$), PR negativity ($P=0.031$) and TNBC molecular subtype ($P=0.009$) (**Table 3**).

Basal characteristics of TNBC

In 132 TNBC cases, 55 (41.7%), 11 (8.3%), 8 (6.1%), 6 (4.6%), 29 (22.0%), and 23 (17.4%)

Table 5. Clinicopathologic characteristics of patients according to triple negative breast cancer subtype

Parameters	*Total (n=132) (%)*	*Basal-like type (n=55) (%)*	*Molecular apocrine type (n=11) (%)*	*Claudine low type (n=8) (%)*	*Immune related type (n=6) (%)*	*Mixed type (n=29) (%)*	*Null type (n=23) (%)*	*P-value*
BRAF V600E								0.610
Negative	128 (97.0)	54 (98.2)	11 (100.0)	8 (100.0)	6 (100.0)	28 (96.6)	21 (91.3)	
Positive	4 (3.0)	1 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.4)	2 (8.7)	
Nuclear BRAF V600E								0.337
Negative	122 (92.4)	53 (96.4)	9 (81.8)	7 (87.5)	6 (100.0)	25 (86.2)	22 (95.7)	
Positive	10 (7.6)	2 (3.6)	2 (18.2)	1 (12.5)	0 (0.0)	4 (13.8)	1 (4.3)	

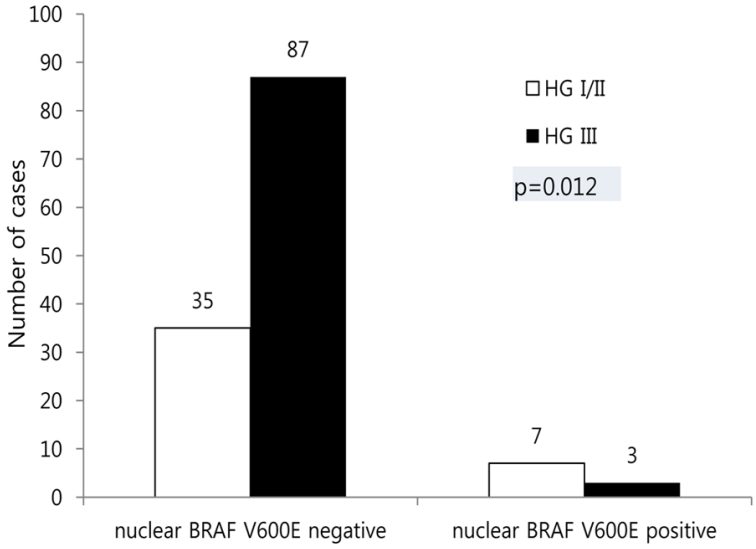

Figure 3. Correlation between BRAF V600E status and clinicopathologic parameters in triple negative breast cancer.

were of basal-like type, molecular apocrine type, claudin low type, immune related type, mixed type and null type, respectively. Lymphocytic infiltration was most frequently found in immune related type (P=0.025), and molecular apocrine type was associated with apocrine differentiation (P=0.026) and lower Ki-67 LI (P=0.005) (**Table 4**).

Expression of BRAF V600E mutation specific antibody in TNBC

In an analysis of immunohistochemical expression of BRAF V600E mutation specific antibody with TNBC, 4 (3.0%) showed cytoplasmic expression (**Figure 2**) and 10 (7.6%) showed nuclear expression. The rate of cytoplasmic BRAF V600E positivity was highest in null type and followed by mixed type and basal-like type, and it was not found in other types of TNBC. The rate of nuclear BRAF V600E positivity was highest in molecular apocrine type, followed by mixed type, claudin low type, null type and basal-like type, and BRAF V600E positive case was not found in immune related type (**Table 5**). The correlation between BRAF V600E status and clinicopathologic parameters was analyzed. The cytoplasmic BRAF V600E status was not associated with clinicopathologic parameters, but the nuclear BRAF V600E status was associated with histological grade (P=0.012). The nuclear BRAF V600E positivity was associated with lower histologic grade (**Figure 3**).

Impact of BRAF V600E status in patient prognosis

A univariate analysis was performed to evaluate the impact of BRAF V600E status on the prognosis of breast cancer and TNBC. The cytoplasmic and nuclear BRAF V600E status did not correlate with shorter disease free survival or overall survival (**Table 6**).

Discussion

In this study, we investigated the BRAF mutation status in breast cancer using IHC with BRAF V600E mutation specific antibody, and 13% of breast cancer and 3% of TNBC showed positivity for BRAF V600E mutation specific antibody. The gold standard method for the detection of BRAF mutation is Sanger sequencing analysis, and other PCR-based methods such as single-strand conformation polymorphism, restriction fragment length polymor-

Table 6. Univariate analysis of the impact of expression in breast cancer on disease-free and overall survival by the log-rank test

Parameter	Number of patients/ recurrence/death	Disease-free survival		Overall survival	
		Mean survival (95% CI) months	*P*-value	Mean survival (95% CI) months	*P*-value
Breast cancer patients group					
BRAF V600E			N/A		0.342
Negative	200/11/17	N/A		64 (63-66)	
Positive	30/0/1	N/A		67 (64-70)	
Nuclear BRAF V600E			0.776		0.817
Negative	213/10/17	66 (65-68)		66 (64-67)	
Positive	17/1/1	65 (63-66)		63 (59-68)	
TNBC patients group					
BRAF V600E			N/A		N/A
Negative	128/14/14	N/A		N/A	
Positive	4/0/0	N/A		N/A	
Nuclear BRAF V600E			N/A		0.987
Negative	122/14/13	N/A		93 (88-98)	
Positive	10/0/1	N/A		69 (60-78)	

Note: TNBC, triple negative breast cancer.

phism (RFLP), mass-array spectrometry, pyrosequencing, and mutation-specific PCR have been also proposed. But these methods require expensive equipment, high technical skills, and have other problems such as tissue heterogeneity, sampling error, suboptimal DNA preservation of formalin-fixed paraffin-embedded (FFPE) tissue. These drawbacks limited its general use in clinical fields [18]. To overcome the abovementioned limitations, novel mouse monoclonal mutation-specific anti-BRAF^{V600E} antibody was developed [6], and it is reported to be very useful in detecting BRAF mutation in various neoplasms [7, 16, 19-21]. Especially in comparison studies using FFPE tissue, the IHC method using BRAF V600E mutation specific antibody was reported to be more specific and more sensitive compared to Sanger sequencing method, suggesting that IHC by BRAF V600E mutation specific antibody could be a new golden standard method [16]. Therefore our results of positive IHC staining of BRAF V600E mutation suggest that BRAF mutation is found in a proportion of breast cancers. In previous studies using sequencing methods, it is reported that 10% of breast cancer cell lines harbored BRAF mutation [9], 3% of breast cancer tissue (IDC) had BRAF mutation, and BRAF mutation was found in 2.6% in TNBC. In our study, a similar rate of BRAF positivity with the previous study regarding TNBC was noted, but in IDC, our study showed a bit higher rate of BRAF mutation compared to the previous study regarding IDC.

In previous studies regarding thyroid cancer tissues, the expression was reported to be diffuse and homogenous in positive cases in general [16, 22], and the result of our study with breast cancer also showed diffuse and homogenous positive staining pattern, which is consistent with previous studies. In this study, the nuclear expression to BRAF V600E mutation specific antibody was found in 8.0% of IDC and 7.6% of TNBC. In previous studies regarding the expression of BRAF V600E mutation specific antibody in other neoplasms, only cytoplasmic expressions are reported [7, 16, 19-22]. In this study, the aberrant nuclear expression for BRAF V600E antibody of breast cancer was associated with clinicopathologic factors such as ER negativity (P=0.003), PR negativity (P=0.031) and TNBC subtype (P=0.009), and it needs to be further evaluated in future studies. On the contrary, the BRAF V600E mutation status did not have association with clinicopathologic factors. In previous studies, BRAF mutation is reported to be associated with several clinicopathologic features related to prognosis, such as lymph node metastasis and distant metastasis in PTC [23], younger age and tumor occurrence from intermittently sun-exposed skin in

malignant melanoma [24, 25], poor prognosis in colon cancer [26, 27], which are different from the result from our study regarding breast cancer. In non-small cell lung cancer, BRAF mutation status was not associated with clinical outcome [28] like the result of our study with breast cancer.

The clinical implication of this study is the potential use of BRAF mutation status for the application of targeted therapy. In various neoplasms including malignant melanoma, drugs targeting BRAF mutation (vemurafenib and dabrafenib) is already in use in preclinical and clinical phase [29-31], so targeted therapy in breast cancers with BRAF mutation can also be considered. Especially in TNBC, which is a small proportion of breast cancer but does not have any effective therapeutic agent except surgery, BRAF mutation targeted therapy can be a possible treatment, and a further study is needed. In conclusion, a proportion of breast cancer and TNBC showed positivity for the IHC by BRAF V600E mutation specific antibody, suggesting BRAF mutation, and BRAF mutation did not show association with clinicopathologic factors.

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Disclosure of conflict of interest

None.

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