Relation between F-18 FDG Uptake of PET/CT and *BRAF*V600E Mutation in Papillary Thyroid Cancer

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Seokho Yoon

Major in Medicine

Department of Medical Sciences

The Graduate School, Ajou University

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Supervised by

Joon-Kee Yoon, M.D., Ph.D.

Major in Medicine

Department of Medical Sciences

The Graduate School, Ajou University

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This certifies that the dissertation of Seokho Yoon is approved.

SUPERVISORY COMMITTEE

Jung Mi Park

Joon-Kee Yoon

Jang-Hee Kim

Young-Sil An

Su Jin Lee

The Graduate School, Ajou University

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- Abstract -

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Purpose: *BRAF*V600E mutation and F-18 fluorodeoxyglucose (FDG) uptake are potential prognostic factors of papillary thyroid cancer (PTC). This study was performed to investigate the relationship between the *BRAF*V600E mutation and F-18 FDG uptake in PTC.

Materials and Methods: We retrospectively included 169 PTC patients who underwent F-18 FDG positron emission tomography/computed tomography (PET/CT) before thyroidectomy from May 2009 to August 2012. Subjects were classified into overt PTC (> 1 cm, n = 76) and papillary thyroid microcarcinoma (PTMC, n = 93) groups. Univariate and multivariate analyses were performed to assess the relationship between maximum standardized uptake value (SUV_{max}) of the primary tumor and clinicopathologic variables.

Results: The *BRAF*V600E mutation was detected in 82.2% (139/169). In all subjects, the *BRAF*V600E mutation and tumor size were independently related to SUV_{max} by multivariate analysis ($P = 0.048$ and $P < 0.001$, respectively). SUV_{max} was significantly higher in tumors with the *BRAF*V600E mutation than in those with wild-type *BRAF* (9.4 ± 10.9 vs. 5.0 ± 4.1, $P < 0.001$). Similarly, in overt PTC group, the *BRAF*V600E mutation and tumor size were independently correlated with SUV_{max} ($P = 0.032$ and $P = 0.001$, respectively). By contrast, in PTMC group, only tumor size was significantly associated with SUV_{max} ($P = 0.010$).

Conclusions: The presence of the *BRAF*V600E mutation is independently associated with high F-18 FDG uptake on preoperative PET/CT in patients with overt PTC, but this relationship was not evident in PTMC. This study provides a better understanding of the relationship between F-18 FDG uptake and *BRAF*V600E mutation in patients with PTC.

Key words: papillary thyroid cancer; *BRAF*V600E mutation; F-18 FDG; PET/CT; SUVmax

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Ⅰ. Introduction

Papillary thyroid cancer (PTC) is the most common histological type of thyroid cancer, accounting for more than 80% of all thyroid malignancies. By virtue of surgical removal of the tumor and radioiodine ablation therapy, well-differentiated PTC has a favorable prognosis, with an overall 5-year survival rate of 95-97% (Xing, 2007; Howlader et al., 2013). However, PTCs with aggressive behavior develop in some patients, often becoming the cause of mortality through tumor recurrence and refractoriness to radioiodine therapy (Sturgeon and Angelos, 2006). For this reason, risk stratification and prognostic evaluation have been the focus of much effort by researchers (Ortiz et al., 2001; Siironen et al., 2005; Tanaka et al., 2005).

Positron emission tomography/computed tomography (PET/CT) with F-18 fluorodeoxyglucose (FDG) is a non-invasive diagnostic tool useful for the evaluation of a variety of malignant tumors (Hustinx et al., 2002; Endo et al., 2006). F-18 FDG PET has been used to locate recurrent thyroid cancers, particularly in cases of elevated serum thyroglobulin concentrations and negative I-131 whole body scintigraphy, because the coincidence of losing radioiodine avidity and gaining the ability to concentrate glucose (the “flip-flop” phenomenon) is observed frequently in differentiated thyroid cancer patients (Feine et al., 1996; Mian et al., 2008). In addition, F-18 FDG PET provides prognostic information. The size of the primary tumor, perithyroidal invasion, lymphovascular invasion and cervical lymph node metastasis are associated with F-18 FDG uptake (Yun et al., 2010; Choi et al., 2011; Kaida et al., 2011; Kim et al., 2012), and larger tumor size is more likely to be associated with higher F-18 FDG uptake (Kim et al., 2012).

The *BRAF* mutations have been found in various cancers including melanoma, colon cancer and thyroid cancer (Davies et al., 2002; Garnett and Marais, 2004). Among the *BRAF* mutations, the *BRAF*V600E mutation, a T1799A point mutation in the B-type Raf kinase gene, is the most common genetic alteration in PTC (Xing, 2007). Similar to F-18 FDG uptake, the *BRAF*V600E mutation has received attention as a potential prognostic factor in PTC (Xing et al., 2005; Riesco-Eizaguirre et al., 2006; Xing et al., 2013).

In recent studies, the possible relationship between the *BRAF*V600E mutation and F-18 FDG uptake has been demonstrated. The *BRAF*V600E mutation was associated with increased

GLUT-1 expression in both primary and metastatic PTCs (Mian et al., 2008). *BRAF* was the most frequently mutated gene in F-18 FDG positive recurrent/metastatic thyroid cancers (Ricarte-Filho et al., 2009). Despite these reports, the relationship between F-18 FDG uptake and the *BRAF*V600E mutation in PTC is still poorly recognized. Therefore, in the present study, we retrospectively evaluated the relationship between the *BRAF*V600E mutation and F-18 FDG uptake of the primary tumor on preoperative PET/CT by analyzing potential clinicopathologic factors affecting F-18 FDG uptake in patients with PTC. Our hypothesis was that PTCs with *BRAF*V600E mutation show more increased F-18 FDG uptake than those with wild-type *BRAF*, and that even small-sized PTCs with the mutation are associated with higher F-18 FDG uptake.

Ⅱ. Materials and methods

A. Patients

Study subjects were recruited through a medical record review from May 2009 to August 2012. During this period, a total of 177 consecutive patients underwent F-18 FDG PET/CT before total thyroidectomy. Of these patients, 1 patient with a final diagnosis of hyalinizing trabecular tumor and 7 patients with the interval from PET/CT to thyroidectomy longer than 7 month were excluded. Ultimately, 169 patients (male:female = 37:132) were included in this retrospective study. Most patients (n = 150) were referred for metastatic workup of thyroid cancers diagnosed by fine needle aspiration, ultrasonography or CT. The rest of the cases (n=19) were incidentalomas detected on PET/CT during the follow-up of other malignancies.

Information on the characteristics of the patients was collected retrospectively by medical record review. Tumor, Node, Metastasis (TNM) stage was determined using the 7th edition of the American Joint Committee on Cancer's Cancer Staging Manual. Tall cell and diffuse sclerosing variants were classified as aggressive histologic types (American Thyroid Association Guidelines Taskforce on Thyroid et al., 2009). A thyroglobulin concentration of 40 ng/mL was used as the cutoff value based on the normal range of a commercial radioimmunoassay kit (Radim, Milan, Italy). Patients were divided into 2 groups according to tumor size: overt PTC (> 1 cm) and papillary thyroid microcarcinoma (≤ 1 cm, PTMC). Clinicopathologic factors affecting F-18 FDG uptake by primary tumors were analyzed with respect to age, sex, tumor size, histologic type, N-M stage, accompanying thyroid disorders affecting the ipsilateral thyroid and preoperative thyroglobulin concentration. Analysis was first performed in all patients with PTC, and then in those with overt PTC or PTMC.

All procedures were in accordance with the ethical standards of our institutional review board on human experimentation (approval no. AJIRB-MED-MDB-12-316). The requirement to obtain informed consent from the patients was waived by our institutional review board.

B. F-18 FDG PET/CT and Image Analysis

Patients were fasted for 6 hours before scanning. The blood glucose concentration was measured to ensure a level below 150 mg/dL. After an intravenous injection of 370 MBq of F-

18 FDG, all patients were instructed to rest comfortably for 60 minutes. Emission PET data were acquired from the base of the skull to the upper thigh in 3-D mode using a Discovery ST scanner (GE Healthcare, Milwaukee, WI / USA), and then they were reconstructed with noncontrast CT (tube rotation time 1 second/revolution, 120 kV, 60 mA, 7.5 mm/rotation, acquisition time 60.9 seconds, scan length 867 mm) by iterative reconstruction (ordered-subsets expectation maximization with 2 iterations and 30 subsets, field of view = 600 mm, slice thickness = 3.27 mm).

For the semi-quantitative analysis, polygonal regions of interest (ROIs) were first drawn on CT images and then copied to attenuation-corrected PET images using the Advantage Workstation (version 4.4, GE Healthcare, Milwaukee, WI / USA). For tumors with a hypermetabolic lesion, ROIs were placed at every transaxial plane of CT images that contained the hypermetabolic lesion. Meanwhile, for those without visually discernible F-18 FDG uptake, ROIs were drawn to cover the whole tumor. In cases of multiple malignant nodules, a ROI was drawn on the largest one. Maximum standardized uptake value (SUV_{max}) was calculated with the injected dose and patient's body weight.

C. *BRAF*V600E Mutation Analysis

DNA was extracted from paraffin-embedded PTC tissue obtained during thyroidectomy. Tumor areas were transferred to an Eppendorf tube and digested with proteinase K (Promega, Madison, WI / USA) at 56˚C for 60 minutes. DNA was isolated using a protein precipitation solution (Qiagen, Hilden / Germany) and isopropanol. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed using a forward (5'-ATAGGTGATTTTGGTCTAGCTCCGG-3') and reverse primer (5'-GATTTTTGTGAATA CTGGGAACT-3'). After amplification and purification, the PCR products were electrophoresed on a 3% TBE-Agarose SFR gel (Amresco, Solon, OH / USA). The gels were photographed under UV trans-illumination using the Gel Doc XR+ System (Bio-Rad, Hercules, CA / USA). 1 band of 189 base pair or two bands of 189/210 base pair was interpreted as the mutation, and 1 band of 210 base pair as the wild-type (Fig. 1). Results were reported within a few days of surgery.

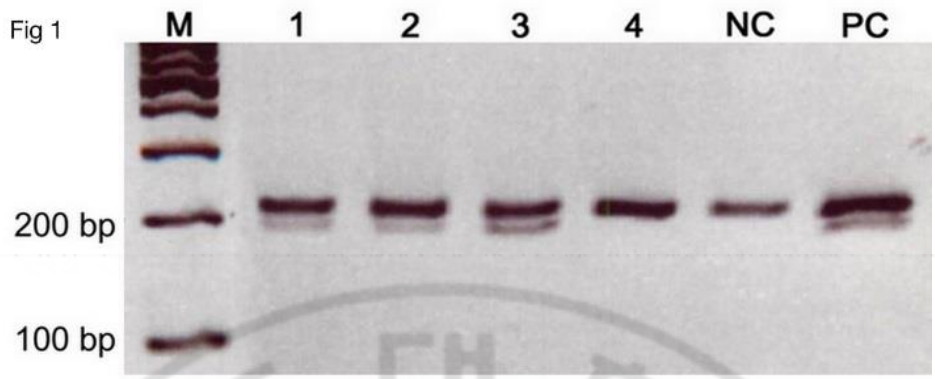

Fig. 1. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to analyze the presence of *BRAF*V600E mutation in papillary thyroid cancer (PTC). Case 1-3 shows 2 bands of 189 and 210 base pair, indicating mutated *BRAF*. Case 4 with 1 band of 210 base pair is wild-type (M: Marker [100-2000 base pair]; 1-4 :DNA extracted from PTC; NC: negative control; PC: positive control).

D. Statistical analysis

All values are presented as the means ± SD. Student's *t* test, the Mann–Whitney test, the Kruskal–Wallis test or one-way ANOVA was used for univariate analysis depending on the types of variables. Logistic regression analysis was used for multivariate analysis of factors associated with SUV_{max}. All statistical analyses were performed using SPSS software (version 22.0; SPSS, Inc., Chicago, IL / USA). A *P* value of less than 0.05 was considered statistically significant.

Ⅲ. Results

A. Patients' Characteristics

The *BRAF*V600E mutation was present in 82.2% of all patients (Table 1). The most common histologic variants were classical and follicular (164/169, 97.0%). The histopathologic examination of surgical specimens detected accompanying thyroid disorders which may increase F-18 FDG uptake in 84 patients (49.7%). All younger patients (≤ 45 years) had TNM stage I disease, whereas the most frequent stage in older patients (> 45 years) was stage III. Average tumor size and SUV_{max} were 1.4 cm and 8.8 g/mL, respectively. Preoperative thyroglobulin was measured in 159 patients and its mean value was 87.9 ng/mL.

B. Relationships between SUV_{max} and Clinicopathologic Variables in All Patients with PTC

The results of univariate and multivariate analyses of clinicopathologic variables that potentially affect F-18 FDG uptake in the whole PTC population are shown in Table 2. The *BRAF*V600E mutation, tumor size, N stage and preoperative thyroglobulin concentration were significantly associated with SUV_{max}. SUV_{max} was significantly higher in the mutated group than in the wild-type group (9.4 ± 10.9 vs. 5.0 ± 4.1, $P < 0.001$, Fig. 2). A pairwise comparison revealed that SUV_{max} was significantly higher in patients with larger tumors (> 2 cm, 20.5 ± 15.2, n = 32) than in those with smaller tumors (< 1 cm, 4.5 ± 4.2, $P < 0.001$; 1-2 cm, 8.6 ± 7.9, $P < 0.001$). Greater extent of lymph node metastasis was also related to a higher SUV_{max} ($P = 0.026$). Patients with increased preoperative thyroglobulin concentrations had higher SUV_{max} values than those with normal thyroglobulin concentrations ($P < 0.001$). On the other hand, age, sex, histological variant, accompanying thyroid disorder and M stage were not significantly associated with SUV_{max}.

A multivariate analysis with all variables included revealed that the *BRAF*V600E mutation ($P = 0.048$) and tumor size ($P < 0.001$) were independent variables affecting F-18 FDG uptake on preoperative PET/CT in patients with PTC.

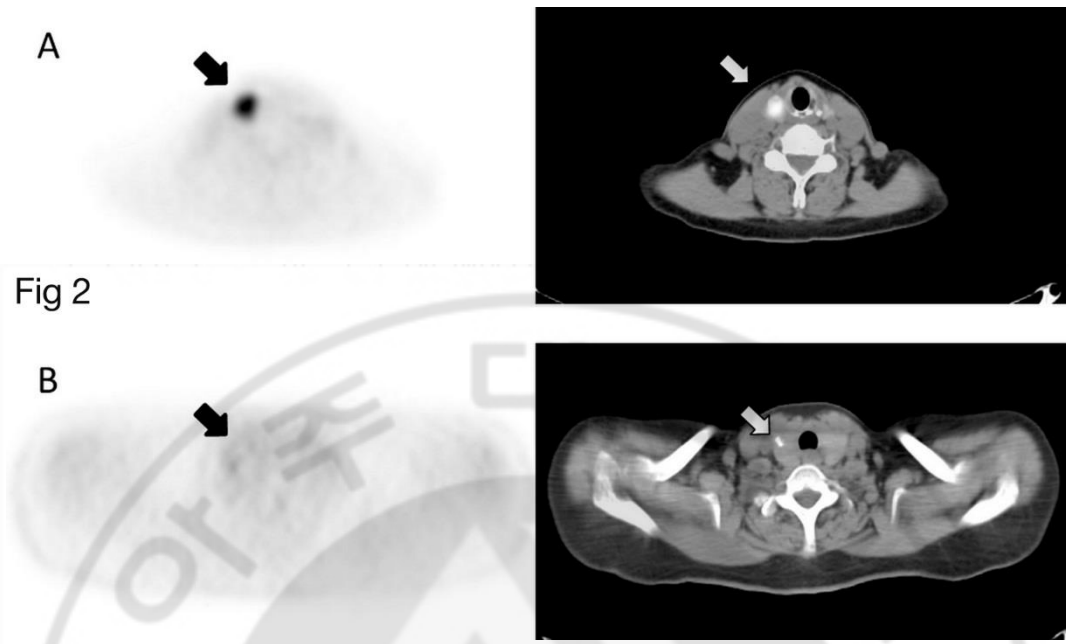

Fig 2

Fig. 2. Representative cases for the relationship between *BRAF*V600E mutation and F-18 fluorodeoxyglucose (FDG) uptake on positron emission tomography/computed tomography (PET/CT). (A) 68-year-old female, SUV_{max} = 20.8, BRAF (+), classical type, T3N1aM0, tumor size = 1.7 cm, thyroglobulin = 90.6 ng/mL, no associated thyroid disease. (B) 60-year-old female, SUV_{max} = 2.2, BRAF(-), classical type, T1bN1aM0, tumor size = 1.7 cm, thyroglobulin = 1.1 ng/mL, thyroiditis. Arrows: thyroid nodules. SUV_{max} = maximum standardized uptake value

C. Relationships between SUV_{max} and Clinicopathologic Variables in Patients with Overt PTC

Similar to the whole PTC population, the *BRAF*V600E mutation was significantly associated with F-18 FDG uptake in overt PTC (Table 3). SUV_{max} was significantly higher in the mutated group than in the wild-type group (15.0 ± 13.6 vs. 6.8 ± 5.3, P = 0.040). There were also significant differences in SUV_{max} depending on the tumor size ($p < 0.001$) and preoperative thyroglobulin concentration (P = 0.039) in this subpopulation. Age, sex, histological variant, accompanying thyroid disorder, N stage and M stage were not associated with SUV_{max}.

Multivariate analysis revealed that the *BRAF*V600E mutation (P = 0.032) and tumor size (P

= 0.001) were independent variables affecting F-18 FDG uptake on preoperative PET/CT in patients with overt PTC.

D. Relationships between SUV_{max} and Clinicopathologic variables in Patients with PTMC

To analyze the relationship between clinicopathologic variables and SUV_{max} in PTMCs, we further categorized by tumor size: ≤ 0.5 and > 0.5 cm. Unlike for the whole PTC and overt PTC populations, the *BRAF*V600E mutation was not significantly associated with SUV_{max} (4.7 ± 4.5 for the mutated group and 3.7 ± 2.5 for the wild-type group, P = 0.784, Table 4). Only tumor size was significantly related to SUV_{max} in both univariate (P = 0.001) and multivariate analyses (P = 0.010). SUV_{max} was higher for PTMCs with a diameter of 5-10 mm than for those with a diameter of 0-5 mm (5.5 ± 4.7 for PTMCs with a diameter of 5-10 mm and 2.5 ± 1.2 for PTMCs with a diameter of 0-5 mm). The other variables, including N stage and preoperative thyroglobulin concentration, were not associated with SUV_{max}.

TABLE 1. Clinicopathologic Characteristics of the Patients

Variables	
Number of patients	169
Age, yr	52 ± 12 (28-81)
Sex, M/F	37/132
*BRAF*V600E mutation, mutated/wild	139/30
Interval from PET/CT to thyroidectomy, d	42 ± 40 (1-203)
Histologic variants, n	
Classical	150
Follicular	14
Tall cell	2
Oncocytic	2
Diffuse sclerosing	1
Accompanying thyroid disorders, n	
Nodular goiter	51
Thyroiditis	22
Adenomatous hyperplasia	3
Nodular goiter with thyroiditis	7
Adenomatous hyperplasia with thyroiditis	1
TNM stage, n	
Age < 45 yr	
I	51
Age ≥ 45 yr	
I	25
II	1
III	64
IVA	25
IVC	3
Tumor size, cm	1.4 ± 1.2 (0.1-8.6)
Preoperative thyroglobulin, ng/mL	87.9 ± 631.1 (0.1-7900.0)*
SUV_{max}, g/mL	8.6 ± 10.2 (1.2-46.5)

Data are presented as mean ± SD (range) or number of patients.
PET/CT = positron emission tomography/computed tomography; SUV_{max} = maximum standardized uptake value.
*n = 159.

TABLE 2. Relationships between SUV_{max} and Clinicopathologic Variables in All Patients with Papillary Thyroid Carcinoma

Variables	n	SUV_{max}	Univariate *P* value	Multivariate *P* value
Age, yr				
≤ 45	51	9.3 ± 11.6	0.569	0.630
> 45	118	8.3 ± 9.6		
Sex				
Male	37	10.9 ± 13.0	0.211	0.568
Female	132	8.0 ± 9.2		
*BRAF*V600E mutation				
Mutated	139	9.4 ± 10.9	< 0.001	0.048
Wild-type	30	5.0 ± 4.1		
Histology				
Non-aggressive	166	8.4 ± 9.8	0.229	0.054
Aggressive	3	22.1 ± 22.0		
Accompanying thyroid disease				
Present	84	7.3 ± 9.0	0.085	0.282
Absent	85	10.0 ± 11.2		
Tumor size, cm				
≤ 1	93	4.5 ± 4.2		
1-2	44	8.6 ± 7.9	< 0.001	< 0.001
2-4	29	20.3 ± 15.0		
> 4	3	22.4 ± 20.9		
N stage				
0	82	6.5 ± 8.0		
1a	44	9.0 ± 9.1	0.026	0.929
1b	43	12.3 ± 13.6		
M stage				
0	166	8.4 ± 9.9	0.120	0.200
1	3	20.7 ± 22.2		
Preoperative thyroglobulin, ng/mL				
≤ 40	130	7.2 ± 8.4	< 0.001	0.366
> 40	29	16.2 ± 14.8		

SUV_{max} = maximum standardized uptake value.

TABLE 3. Relationships between SUV_{max} and Clinicopathologic Variables in Patients with Overt Papillary Thyroid Carcinoma

Variables	n	SUV_{max}	Univariate P value	Multivariate P value
Age, yr				
≤ 45	21	16.4 ± 14.7	0.387	0.724
> 45	55	12.5 ± 12.1		
Sex				
Male	18	17.2 ± 15.6	0.282	0.914
Female	58	12.5 ± 11.9		
BRAFV600E mutation				
Mutated	63	15.0 ± 13.6	0.040	0.032
Wild-type	13	6.8 ± 5.3		
Histology				
Non-aggressive	73	13.3 ± 12.5	0.542	0.148
Aggressive	3	22.1 ± 22.0		
Accompanying thyroid disease				
Present	33	11.5 ± 12.6	0.205	0.278
Absent	43	15.3 ± 13.0		
Tumor size, cm				
1-2	44	8.6 ± 7.9		
2-4	29	20.3 ± 15.0	< 0.001	0.001
> 4	3	22.4 ± 20.9		
N stage				
0	24	11.7 ± 11.9		
1a	21	13.3 ± 11.1	0.662	0.841
1b	31	15.3 ± 14.8		
M stage				
0	73	13.3 ± 12.5	0.491	0.822
1	3	20.7 ± 22.2		
Preoperative thyroglobulin, ng/mL				
≤ 40	49	11.5 ± 11.4	0.039	0.403
> 40	25	18.3 ± 15.0		

SUV_{max} = maximum standardized uptake value.

TABLE 4. Relationships between SUV_{max} and Clinicopathologic Variables in Patients with Papillary Thyroid Microcarcinoma

Variables	n	SUV_{max}	Univariate P value	Multivariate P value
Age, yr				
≤ 45	30	4.3 ± 4.3	0.740	0.980
> 45	63	4.6 ± 4.1		
Sex				
Male	19	4.9 ± 5.4	0.396	0.877
Female	74	4.5 ± 3.9		
BRAFV600E mutation				
Mutated	76	4.7 ± 4.5	0.784	0.568
Wild-type	17	3.7 ± 2.5		
Histology				
Non-aggressive	93	4.5 ± 4.2		
Aggressive	0			
Accompanying thyroid disease				
Present	51	4.5 ± 3.7	0.997	0.907
Absent	42	4.5 ± 4.8		
Tumor size, cm				
≤ 0.5	29	2.5 ± 1.2	0.001	0.010
> 0.5	64	5.5 ± 4.7		
N stage				
0	58	4.3 ± 4.2		
1a	23	5.1 ± 4.1	0.198	0.680
1b	13	5.2 ± 5.1		
M stage				
0	93	4.5 ± 4.2		
1	0			
Preoperative thyroglobulin, ng/mL				
≤ 40	81	4.6 ± 4.3	0.682	0.935
> 40	4	5.1 ± 4.6		

SUV_{max} = maximum standardized

Ⅳ. Discussion

This study was performed to investigate the relationship between the *BRAF*V600E mutation and F-18 FDG uptake on preoperative PET/CT in patients with PTC. The *BRAF*V600E mutation was independently related to SUV_{max} in a multivariate analysis that included various clinicopathologic factors. In subgroup analyses, this relationship persisted in overt PTC, but not in PTMC. These results indicate that 2 potential prognostic factors, the *BRAF*V600E mutation and F-18 FDG uptake by the primary tumor, are closely related in patients with overt PTC.

Many studies have demonstrated that the *BRAF*V600E mutation is related to tumor aggressiveness and poor prognosis in PTC (Xing et al., 2005; Riesco-Eizaguirre et al., 2006; Xing et al., 2013). The *BRAF*V600E mutation was independently related to known prognostic factors such as extrathyroidal invasion, lymph node metastasis, advanced tumor stage (III/IV) and aggressive subtypes. The presence of the *BRAF*V600E mutation was associated with the recurrence of PTC, even in a low-risk group (Xing et al., 2005). In a retrospective multicenter study, *BRAF*V600E mutation-positive patients experienced more deaths per 1,000 person-years than their wild-type counterparts (11.80 vs. 2.25, Hazards Ratio = 3.53) (Xing et al., 2013).

Like the *BRAF*V600E mutation, F-18 FDG uptake is also accepted as a potential prognostic factor in thyroid cancer. F-18 FDG positivity, SUV_{max} (> 10 g/mL) and metabolic tumor volume (> 125 mL) were significantly correlated with survival regardless of radioiodine avidity (Wang et al., 2000). Survival of stage I-III patients with positive F-18 FDG uptake was as poor as that of stage IV thyroid cancer patients (Robbins et al., 2006).

Besides the role as a prognostic factor, there is a similarity between F-18 FDG uptake and the *BRAF*V600E mutation regarding the loss of radioiodine avidity. Similar to the “flip-flop” phenomenon of F-18 FDG uptake, in recurrent thyroid cancers, most (79%) of the I-131 negative group had the *BRAF*V600E mutation, while most (82%) of the I-131 positive group was wild-type (Barollo et al., 2010). Similarly, in patients with metastatic PTC, the I-131 negative group had a higher rate of *BRAF*V600E mutation (77%) than did the I-131 positive group (43%) (Mian et al., 2008). From the perspective of molecular changes, the *BRAF*V600E

mutation in PTC is related to the silencing of thyroid iodide-metabolizing genes (Durante et al., 2007; Mian et al., 2008), and also impairs the targeting of sodium/iodide symporter to the cell membrane (Riesco-Eizaguirre et al., 2006). Considering those previous reports, it is reasonable that in the present study the *BRAF*V600E mutation was associated with F-18 FDG uptake on preoperative PET/CT in PTC.

F-18 FDG uptake is based on enhanced aerobic glycolysis in cancer cells, known as Warburg effect (Warburg, 1956). There are some experimental evidence that *BRAF* mutation is involved in increased glycolysis in PTC. Mitochondrial localization of *BRAF*V600E led to the reduction of mitochondrial O_2 consumption and increased glucose uptake in PTC (Lee et al., 2011). The expression of the M2 isoform of pyruvate kinase, which is the rate-limiting step of glycolysis, was also significantly higher in PTCs harboring *BRAF*V600E (Christofk et al., 2008; Feng et al., 2013). Meanwhile, GLUT1, GLUT3 and hexokinase II play an important role in the trapping of F-18 FDG by cancer cells (Pauwels et al., 1998). At the transcriptional level, hypoxia-inducible factor (HIF)-1 exerts influence on glycolytic shift in cancer cells by targeting GLUT1, GLUT3 and hexokinase II (Denko, 2008; Bensinger and Christofk, 2012). Several studies demonstrated that GLUT1 expression and HIF-1α level were significantly higher in *BRAF*-mutated PTC compared to *BRAF*-wild type PTC (Durante et al., 2007; Mian et al., 2008; Zerilli et al., 2010; Grabellus et al., 2012). HIF-1 can be upregulated via mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway to increase glycolysis (Munoz-Pinedo et al., 2012; Xing, 2013). This Ras-Raf-MEK-ERK pathway is aberrantly activated by the mutation of *BRAF* gene (Davies et al., 2002; Garnett and Marais, 2004) and has an important role in the tumorigenesis in PTC (Xing, 2013). Therefore, the induction of MAPK pathway by *BRAF* mutation and the subsequent activation of HIF-1 resulting in increased glycolysis may explain the mechanism behind the association between F-18 FDG uptake and *BRAF*V600E mutation.

Clinicopathologic factors influencing F-18 FDG positivity of PTC on preoperative PET/CT have been previously evaluated by other researchers (Kim et al., 2012). Univariate analysis revealed that size, cervical lymph node metastasis and TNM stage were significantly correlated with F-18 FDG positivity. A multivariate analysis revealed that size and cervical lymph node metastasis were independent predictors of F-18 FDG positivity. Compared with this previous report, our study provided additional insight into the relationship between the

*BRAF*V600E mutation and SUV_{max} on PET/CT in PTC.

From a clinical perspective, PTMC is noteworthy in that most cases are detected incidentally, and aggressive behavior is not uncommon (Lee et al., 2006). In the present study, an analysis with the PTMC subpopulation (93/169, 55%) showed that tumor size was significantly associated with SUV_{max}. This result was in accordance with a few previous reports. Jeong et al. performed a prospective study evaluating 44 PTMC patients; SUV from PET imaging was correlated with tumor size only among various clinicopathologic factors (Jeong et al., 2006). Hwang et al. showed that visually identifiable F-18 FDG uptake was dependent on tumor size and the presence of Hashimoto thyroiditis (Hwang et al., 2014). Yun et al. also found a moderate dependency of SUV on tumor size of PTMC (Yun et al., 2010). Other than the size dependency of tumor metabolism itself, there is another contributing factor: the "partial volume effect" in PET/CT imaging. The partial volume effect leads to underestimation of the F-18 FDG uptake in small tumors (Soret et al., 2007), and it may have influenced the significant positive correlation between SUV_{max} and tumor size in the present study.

In contrast to tumor size, the *BRAF*V600E mutation, in the present study, was not correlated with SUV_{max} in PTMC. According to the literatures, there is a controversy over the clinical significance of the *BRAF*V600E mutation in PTMC. Lee et al. reported that the prevalence of *BRAF*V600E mutation was significantly higher in aggressive PTMC than in nonaggressive tumors (Lee et al., 2009). Virk et al. found that the *BRAF*V600E mutation occurred during an early stage of carcinogenesis and was associated with extrathyroidal invasion and lymph node metastasis (Virk et al., 2013). On the contrary, Zheng et al. found that the *BRAF*V600E mutation was not associated with tumor recurrence in PTMC (Zheng et al., 2013), and a study by Walczyk et al. demonstrated no correlation between the *BRAF*V600E mutation and tumor aggressiveness or recurrence in PTMC after a 12-year clinical follow-up (Walczyk et al., 2014). In a couple of studies with Korean population, which has a high prevalence of *BRAF*V600E mutation, no significant relationships were found between the *BRAF*V600E mutation and clinicopathologic factors (Kim et al., 2005; Choi et al., 2013). Therefore, the role of the *BRAF*V600E mutation as a prognostic factor in PTMC is still uncertain, and our result in PTMC supports those negative results.

There were a few limitations in this work. We retrospectively analyzed PTC patients who had undergone preoperative F-18 FDG PET/CT and subsequent thyroidectomy. We set the

maximum interval between PET/CT and surgery to 7 months. Although PTCs are typically slow-growing, there can be a change in F-18 FDG uptake in some tumors. In addition, as we mentioned above, the partial volume effect may have influenced F-18 FDG uptake in PTMC. However, we were not able to perform partial volume correction, because the necessary in-house software has not been developed yet. Therefore, a further analysis implementing partial volume correction is required for the PTMC subpopulation to clarify the association between *BRAF*V600E mutation and F-18 FDG uptake in PTC.

Ⅴ. Conclusion

2 potential prognostic factors of PTC, the *BRAF*V600E mutation and F-18 FDG uptake, are closely related in overt PTC, but not in PTMC. The present study affords a better understanding of the relationship between the *BRAF*V600E mutation and F-18 FDG uptake in PTC. In addition, clinicians should notice that the clinical significance of F-18 FDG in PTC can be different between overt PTC and PTMC because of the technical limitation of PET/CT.

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– 국문요약 –

유두상 갑상선암에서 PET/CT의 F-18 FDG 섭취와 *BRAF*V600E mutation의 연관성

아주대학교 대학원 의학과

윤 석 호

(지도교수 : 윤준기)

목적: *BRAF*V600E mutation과 F-18 fluorodeoxyglucose (FDG) 섭취는 유두상 갑상선암 (papillary thyroid cancer, PTC)의 잠재적인 예후인자이다. 본 연구는 유두상 감상선암에서 *BRAF*V600E mutation과 F-18 FDG 섭취의 연관성을 조사하기 위하여 시행되었다.

방법: 2009년 9월부터 2012년 8월까지 갑상선 전절제술을 받기 전에 positron emission tomography/computed tomography (PET/CT)를 시행받은 169명의 갑상선 유두상암 환자를 대상으로 후향적인 연구를 시행하였다. 대상자는 over PTC (> 1 cm, n = 76)와 papillary thyroid microcarcinoma (PTMC, n = 93)의 두 집단으로 분류하였다. 원발성 종양의 maximum standardized uptake value (SUV_{max})와 임상적, 병리학적 변수들의 관계를 조사하기 위하여 단변량 분석과 다변량 분석을 시행하였다.

결과: *BRAF*V600E mutation은 82.2% (139/169)의 환자에서 발견되었다. 다변량 분석상 모든 대상자들에서 *BRAF*V600E mutation (P = 0.048)과 종양 크기 (P < 0.001)는 독립적으로 SUV_{max}와 유의한 연관성을 보였다. SUV_{max}는 wild-type *BRAF*인 종양에서보다 *BRAF*V600E mutation인 종양에서 유의하게 높았다 (9.4 ± 10.9 vs. 5.0 ± 4.1, P < 0.001). Overt PTC 집단에서 *BRAF*V600E mutation (P = 0.032)과 종양 크기 (P = 0.001)는 독립적으로 SUV_{max}와 유의한 연관성을 보였다. 그러나 PTMC 집단에서는 종양 크기만이

SUV_{max}와 유의한 연관성을 보였다 ($P = 0.010$).

결론: 크기가 1 cm보다 큰 유두상 갑상선암에서 *BRAF*V600E mutation의 존재는 수술 전 PET/CT상 높은 F-18 FDG 섭취와 독립적으로 유의한 연관성을 보였으나, 1 cm보다 작은 유두상 갑상선암에서는 유의한 연관성을 보이지 않았다. 본 연구는 유두상 갑상선암 환자에서 *BRAF*V600E mutation과 F-18 FDG 섭취의 연관성에 대한 증거를 제시하였다.

핵심어: 유두상 갑상선암; *BRAF*V600E mutation; F-18 FDG; PET/CT; SUVmax