Association Between ^{18}F -FDG Avidity and the BRAF Mutation in Papillary Thyroid Carcinoma

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Received: 16 April 2015 / Revised: 2 September 2015 / Accepted: 4 September 2015 / Published online: 22 September 2015
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Abstract

Purpose The BRAF mutation, a potential prognostic factor in papillary thyroid carcinoma (PTC), is associated with a high expression of the glucose transporter gene. We investigated which clinicopathologic factors, including BRAF mutation status, influence ^{18}F -fluoro-2-deoxyglucose (^{18}F -FDG) avidity.

Methods We retrospectively reviewed 55 patients who underwent BRAF analysis from biopsy-confirmed PTC and ^{18}F -FDG positron emission tomography/computed tomography within 6 months before undergoing thyroid surgery from September 2008 to August 2014. Tumors were considered to be ^{18}F -FDG avid if the uptake was greater than that of the liver. ^{18}F -FDG uptake of PTCs was also analyzed semiquantitatively using SUV_{max} . The association between ^{18}F -FDG avidity and clinicopathologic variables (age, tumor size, perithyroidal extension, cervical lymph node status, and BRAF mutation status) was investigated.

Results Twenty-nine (52.7 %) of 55 patients had ^{18}F -FDG-avid PTCs. PTCs with the BRAF mutation showed higher ^{18}F -FDG avidity (24/38, 63.2 %) than those without (5/17, 29.4 %). The BRAF mutation ($p=0.025$) and tumor size ($p=0.003$) were significantly associated with ^{18}F -FDG avidity in univariate analysis, and the BRAF mutation status remained significant after adjusting for tumor size in multivariate

analysis ($p=0.015$). In the subgroup of tumor size ≥ 1 cm, the BRAF mutation was the only factor significantly associated with ^{18}F -FDG avidity ($p=0.021$). The mean SUV_{max} of PTCs with the BRAF mutation was significantly higher than that of those without (4.89 ± 6.12 vs. 1.96 ± 1.10 , $p=0.039$).

Conclusions The BRAF mutation must be one of the most important factors influencing ^{18}F -FDG avidity in PTCs, especially in those with a tumor size ≥ 1 cm.

Keywords ^{18}F -fluorodeoxyglucose · Positron-emission tomography · BRAF mutation · Papillary thyroid carcinoma

Introduction

Papillary thyroid carcinoma (PTC) shows variable ^{18}F -fluoro-2-deoxyglucose (^{18}F -FDG) uptake. Many studies have attempted to identify factors associated with ^{18}F -FDG uptake in PTC, with tumor size associated with positive ^{18}F -FDG uptake [1–3]. However, reports regarding the associations of other factors and the ^{18}F -FDG uptake are inconsistent. Several studies have shown that the ^{18}F -FDG avidity of PTC is related to known poor prognosis factors [4], such as lymph node metastasis [2, 5] and perithyroidal extension [5], although there are conflicting results [1–3, 6].

Variable degrees of ^{18}F -FDG uptake may also be explained by the presence of the BRAF mutation. The BRAF mutation is one of the most common mutations in differentiated thyroid cancer (approximately 45 % of sporadic PTCs) [7] and one of the factors influencing PTC prognosis [8]. The BRAF mutation is related to the RAS-RAF-MEK-ERK signaling pathway (MAPK pathway), which is generally activated by normal signaling and causes normal cell growth. However, in the presence of the BRAF mutation, the MAPK pathway is aberrantly activated, resulting in tumorigenesis [9]. The MAPK

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pathway also stimulates the expression of the GLUT-1 transporter gene [10], thereby suggesting that BRAF mutation-positive PTC may have increased ^{18}F -FDG uptake.

It has been suggested that functionally more differentiated thyroid cancer with retained iodine uptake and normal low glucose metabolism can later change to dedifferentiated cancer with increased glucose metabolism and loss of iodine uptake [11]. A recent report also showed that relatively well differentiated PTCs have high expression of iodine metabolism-related genes, such as sodium/iodide symporter and pendrin genes, whereas less differentiated PTCs have high expression of the GLUT-1 transporter gene [12]. However, this ‘flip-flop’ phenomenon, which refers to the inverse correlation between iodine uptake and ^{18}F -FDG uptake [11], can also be explained by the BRAF mutation. BRAF mutant PTC is associated with low expression of iodine metabolism-related genes and high expression of GLUT-1 [13]. The BRAF mutation has also been related to a negative ^{131}I uptake and positive ^{18}F -FDG uptake in recurrent PTC patients [14].

Based on the previous studies, we hypothesized that the BRAF mutation may increase the ^{18}F -FDG uptake in PTC by activating the MAPK pathway and in turn stimulating glucose transport. However, the association between the BRAF mutation and ^{18}F -FDG avidity has not been well studied,

particularly in the preoperative condition. Therefore, we investigated which clinicopathologic factors, including the BRAF mutation status, influence ^{18}F -FDG avidity.

Materials and Methods

Study Subjects

With approval from the Institutional Review Board of Asan Medical Center (IRB No. 2014–1002), we retrospectively reviewed 55 consecutive patients with PTC who underwent BRAF analysis by PCR analysis of biopsy-confirmed PTC and preoperative ^{18}F -FDG PET/CT imaging within 6 months before thyroid surgery among those patients who underwent thyroid surgery between September 2008 and August 2014. All patients underwent either total thyroidectomy ($n=34$) or unilateral thyroid lobectomy ($n=21$). Of these patients, 40 had central neck dissection, 11 had modified radical neck dissection, and four had no neck dissection. The reasons for performing a ^{18}F -FDG PET/CT scan were a preoperative work-up of PTC ($n=39$) or other malignancy work-up ($n=16$). A summary of the patient characteristics is shown in Table 1.

Table 1 Patient characteristics

Characteristics	Total patients ($n=55$)	Staging work-up ($n=39$)	Other malignancy work-up ($n=16$)	p value
Age at diagnosis (year, mean $\pm$ SD)	49.4 $\pm$ 13.6	46.1 $\pm$ 12.7	57.4 $\pm$ 12.4	0.004*
Sex				
Female, n (%)	40 (72.7 %)	29 (74.4 %)	11 (68.8 %)	0.744
Male, n (%)	15 (27.3 %)	10 (25.6 %)	5 (31.3 %)	
Surgery				
Total thyroidectomy, n (%)	34 (61.8 %)	23 (59.0 %)	11 (68.8 %)	0.556
Unilateral thyroid lobectomy, n (%)	21 (38.2 %)	16 (41.0 %)	5 (31.3 %)	
Lymph node dissection				
Central neck dissection, n (%)	40 (72.7 %)	28 (71.8 %)	12 (75.0 %)	0.001*
Modified radical neck dissection, n (%)	11 (20.0 %)	11 (28.2 %)	0 (0 %)	
No neck dissection, n (%)	4 (7.3 %)	0 (0 %)	4 (25.0 %)	
Tumor size (cm, mean $\pm$ SD)	1.24 $\pm$ 0.94	1.29 $\pm$ 1.03	1.13 $\pm$ 0.71	0.609
Perithyroidal extension, n (%)	28 (50.9 %)	23 (59.0 %)	5 (31.3 %)	0.080
Cervical lymph node metastasis, n (%)	35 (63.6 %)	29 (74.4 %)	6 (37.5 %)	0.014*
AJCC stage				
Stage I, n (%)	35 (63.6 %)	26 (66.7 %)	9 (56.3 %)	0.194
Stage II, n (%)	4 (7.3 %)	2 (5.1 %)	2 (12.5 %)	
Stage III, n (%)	11 (20.0 %)	6 (15.4 %)	5 (31.3 %)	
Stage IV, n (%)	5 (9.1 %)	5 (12.8 %)	0 (0 %)	
BRAF mutation, n (%)	38 (69.1 %)	26 (66.7 %)	12 (75.0 %)	0.750

SD standard deviation, AJCC American Joint Committee on Cancer, PTC papillary thyroid carcinoma

* $p<0.05$ was considered significant

BRAF Mutation Analysis: DNA Isolation and Direct DNA Sequencing

DNA was extracted from formalin-fixed, paraffin-embedded PTC tissue obtained from core needle biopsy ($n=36$), thyroidectomy ($n=8$), or percutaneous needle aspiration ($n=11$) specimens. All specimens were histologically confirmed as PTC.

All specimens were washed into collection tubes with 1 mL of normal saline. Next, 0.5 mL of lysis buffer (100 mmol/L sodium chloride; 10 mmol/L tris [hydroxymethyl] aminomethane [Tris], pH 8.0; 10 mmol/L EDTA, pH 8.0; and 0.5 % [wt/vol] sodium dodecyl sulphate) and 7 μ L of proteinase K (20 mg/mL; Gibco BRL, Carlsbad, CA, USA) were added to each sample, and the samples were incubated at 55 °C for 12–18 h, followed by 10 min at 95 °C in order to eliminate any remaining proteinase K activity. Genomic DNA was purified using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany).

For BRAF V600E mutation analysis, exon 15 of the BRAF gene was PCR amplified using the following primer set: 5'-TGCTTGCTCT GATAGGAAAA TG-3' (forward) and 5'-CTGATGGGAC CCACTCCAT-3' (reverse). The amplification protocol consisted of initial denaturation at 94 °C for 2 min, 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, and extension at 68 °C for 40 s, followed by a final extension at 68 °C for 7 min using KOD FX Taq DNA polymerase (Toyobo, Osaka, Japan). A single major PCR product was confirmed by electrophoresis of each PCR product on a 2.5 % (wt/vol) agarose gel. PCR products were subsequently sequenced using the forward primer described above and the Big Dye Terminator (ABI Systems, Applied Biosystems, Foster City, CA). Each DNA sequence was read on an ABI-PRISM 3100 automatic sequencer (Applied Biosystems) in order to determine the presence of the BRAF mutation.

¹⁸F-FDG PET/CT Acquisition

All patients were instructed to fast for at least 6 h and were given 5.18–7.4 MBq/kg (0.14–0.2 mCi/kg) of ¹⁸F-FDG after measuring their blood glucose level. Blood glucose levels were controlled in order to remain below 8.33 mmol/L (150 mg/dL). Imaging acquisition started 50–70 min after the ¹⁸F-FDG injection, using one of the following scanners: Biograph Sensation 16 ($n=5$; Siemens, Knoxville, TN, USA), Biograph TruePoint 40 ($n=13$; Siemens), Discovery STe 8 ($n=3$; GE Healthcare, Milwaukee, WI, USA), Discovery 690 ($n=12$; GE Healthcare), Discovery 690 Elite ($n=13$; GE Healthcare) or Discovery 710 ($n=9$; GE Healthcare). PET images were acquired from the skull base to the mid-thigh in 3D mode and with five to eight bed positions of 2–3 min each, depending on the PET/CT scanner. All PET images were

reconstructed using an iterative algorithm with attenuation correction with CT images.

PET/CT Analysis

The avidity and location of the focal ¹⁸F-FDG uptake in the thyroid seen on PET/CT images were visually analyzed by two experienced nuclear physicians (S.H.L. and S.H.) who were blinded to the patients' clinical information. When they were in disagreement, the cases were referred to a third, nuclear medicine board-certified physician (S.Y.C.). ¹⁸F-FDG uptake of PTCs that was greater than that of the hepatic activity was considered to be ¹⁸F-FDG avid, and equal to or less than that of hepatic activity was considered ¹⁸F-FDG non-avid. If multifocal uptakes were present, only the lesion in which the BRAF mutation analysis was performed was used for statistical analysis. ¹⁸F-FDG uptake of PTCs was also analyzed semiquantitatively using SUV_{max}. Because of the varying spatial resolution of the six scanners used in this study, voxel resampling and SUV harmonization were performed in order to increase the inter-scanner comparability of SUV_{max}. Briefly, voxel sizes of PET images obtained on different scanners were made equal by image interpolation resampling ($5 \times 5 \times 5$ -mm³ voxels), and SUV was harmonized by matching the recovery coefficient profiles between scanners, according to the method described by Lasnon et al. [15].

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (version 17.0; SPSS Inc., Chicago, IL, USA). To evaluate the inter-observer variability of the ¹⁸F-FDG avidity analysis, kappa statistics were used. Univariate analyses were performed in order to identify any possible association between ¹⁸F-FDG avidity and six independent variables using univariate binary logistic regression analysis: age (< 45 or $\geq$ 45 years), sex, tumor size (< 1 or $\geq$ 1 cm), perithyroidal extension, lymph node metastasis, and BRAF mutation status (positive or negative). Variables with $p < 0.05$ in the univariate analysis were further analyzed using multivariate logistic regression analysis. Univariate and multivariate linear regression analysis was performed analogously, using harmonized SUV_{max} as a dependent variable and the aforementioned six potential predictors as independent variables. The Mann–Whitney U test or Fisher's exact test was used to compare characteristics or means of the harmonized SUV_{max} in the subgroups.

For the subgroup with tumor $\geq$ 1 cm, tumor size as a continuous variable and the BRAF mutation status were entered into the multivariate logistic regression model in order to evaluate their independent contribution to ¹⁸F-FDG avidity. A p value less than 0.05 was considered significant in all tests.

Results

Among the 55 patients included in this study, 29 (52.7 %) had ^{18}F -FDG-avid PTCs. The proportion of ^{18}F -FDG avidity did not differ significantly in the preoperative work-up group and the other malignancy work-up group [46.2 % (18/39) vs. 68.8 % (11/16), $p=0.149$]. Interobserver agreement for visual analysis of ^{18}F -FDG avidity was 90.9 % (kappa=0.819, $p<0.001$). PTCs with the BRAF mutation showed a higher proportion of ^{18}F -FDG avidity (24/38, 63.2 %) than those without the BRAF mutation (5/17, 29.4 %). Figure 1 shows the representative ^{18}F -FDG PET images of PTCs with and without the BRAF mutation.

Table 2 shows the results of univariate and multivariate logistic regression analyses of the factors associated with ^{18}F -FDG avidity. Among the tested variables, tumor size and BRAF mutation status were significantly associated with ^{18}F -FDG avidity on univariate analysis ($p=0.003$ and 0.025 , respectively). Both the tumor size and the BRAF mutation status remained significant independent variables in a multivariate logistic regression analysis ($p=0.002$ and 0.015 , respectively). Similar results were obtained when we performed a subgroup analysis staging work-up patients only; both tumor size and BRAF mutation were significantly associated with ^{18}F -FDG avidity on univariate ($p=0.004$ and 0.049 , respectively) and multivariate ($p=0.003$ and 0.022 , respectively) analyses.

When the ^{18}F -FDG uptake of PTCs was analyzed semiquantitatively using harmonized SUV_{max} , the mean SUV_{max} of the BRAF mutation-positive tumors was significantly higher than that of the BRAF mutation-negative tumors (4.89 ± 6.12 vs. 1.96 ± 1.10 , $p=0.039$, Fig. 2). On univariate linear regression analysis, the tumor size was significantly associated with the SUV_{max} ($p<0.001$), and both the tumor size and the BRAF mutation were significantly associated with the SUV_{max} in multivariate linear regression analyses ($p<0.001$ and $p=0.012$, respectively; Table 3). Using SUV_{max} with a cut-off 5.2, the positive predictive value of the BRAF mutation of PTC was 100 %.

As the proportion of ^{18}F -FDG avidity differed significantly according to tumor size, we performed subgroup analysis (< 1 and ≥ 1 cm) considering the PET resolution. The BRAF mutation positivity was not significantly different between the two groups [69.2 % (18/26) vs. 69.0 % (20/29), $p=0.983$]. In the subgroup with tumor size < 1 cm, neither tumor size nor BRAF mutation was significantly associated with ^{18}F -FDG avidity or SUV_{max} . In comparison, in the subgroup with tumor size ≥ 1 cm, the BRAF mutation was significantly associated with the ^{18}F -FDG avidity seen in the multivariate logistic regression analyses ($p=0.021$, Table 4). Both tumor size and the BRAF mutation were significantly associated with the SUV_{max} ($p<0.001$ and $p=0.012$, respectively).

Fig. 1 Whole-body maximum intensity projection ^{18}F -FDG PET images of representative cases. **a** A 0.6-cm-sized PTC with BRAF mutation in a 56-year-old man showing high ^{18}F -FDG uptake ($\text{SUV}_{\text{max}}=3.2$). **b** A 1.6-cm-sized PTC without BRAF mutation in a 71-year-old woman and revealing non- ^{18}F -FDG avidity

Table 2 Univariate and multivariate logistic regression analysis of the factors associated with ^{18}F -FDG avidity ($n=55$)

Characteristics	¹⁸ F-FDG avidity	Univariate analysis		Multivariate analysis	
		OR (95 % CI)	<i>p</i> value	OR (95 % CI)	<i>p</i> value
Age					
<45	11/26 (42.3 %)				
≥45	18/29 (62.1 %)	2.231 (0.757–6.576)	0.145		
Sex					
Female	22/40 (55.0 %)				
Male	7/15 (46.7 %)	0.716 (0.218–2.354)	0.582		
Size					
<1 cm	8/26 (30.8 %)				
≥1 cm	21/29 (72.4 %)	5.906 (1.843–18.931)	0.003*	7.875 (2.102–29.505)	0.002*
Perithyroidal extension					
No	13/27 (48.1 %)				
Yes	16/28 (57.1 %)	1.436 (0.496–4.157)	0.505		
Cervical lymph node metastasis					
No	9/20 (45.0 %)				
Yes	20/35 (57.1 %)	1.630 (0.539–4.927)	0.387		
BRAF mutation					
No	5/17 (29.4 %)				
Yes	24/38 (63.2 %)	4.114 (1.198–14.133)	0.025*	6.000 (1.414–25.459)	0.015*
Total	29/55 (52.7 %)				

OR odds ratio, CI confidence interval

* $p<0.05$ was considered significant

Discussion

Our study findings show that the BRAF mutation is the only significant factor associated with ^{18}F -FDG avidity in PTCs ≥ 1 cm in size. This result supports the hypothesis that the BRAF mutation may increase ^{18}F -FDG uptake in PTC by activating the MAPK pathway and in turn stimulating glucose transport. In the initial multivariate analysis, among the various clinicopathologic factors, tumor size and BRAF mutation status were significantly associated with ^{18}F -FDG avidity. Several studies have shown that the ^{18}F -FDG uptake of PTCs

is significantly associated with tumor size [1–3], similar to our study. However, when we performed subgroup analyses in two groups defined by tumor size (< 1 and ≥ 1 cm, considering the PET resolution), the BRAF mutation status was the only factor significantly associated with ^{18}F -FDG avidity in the larger size tumor subgroup (≥ 1 cm), whereas the association between tumor size and avidity was not significant in the two subgroups. The BRAF mutation status was not a significant factor in the smaller size subgroup (< 1 cm). This result may be due to underestimating the ^{18}F -FDG uptake in small tumors due to a partial volume effect [16]. The partial volume

Fig. 2 Harmonized SUV_{max} of the BRAF wild-type and BRAF mutant PTCs. **a** The mean SUV_{max} of the BRAF mutant tumors was significantly higher than that of the BRAF wild-type tumors (4.89 ± 6.12 vs. $1.96 \pm$

1.10 , $p=0.039$). **b** In the patient subgroup with tumors size ≥ 1 cm, the mean SUV_{max} of the BRAF mutant tumors was significantly higher than that of the BRAF wild-type tumors (7.57 ± 7.49 vs. 2.43 ± 1.34 , $p=0.034$)

Table 3 Univariate and multivariate linear regression analysis of the factors associated with harmonized SUV_{max} (*n*=55)

Characteristics	Univariate analysis		Multivariate analysis	
	B (95 % CI)	<i>p</i> value	B (95 % CI)	<i>p</i> value
Age	0.090 (−0.015–0.194)	0.091		
Sex	0.907 (−2.320–4.133)	0.575		
Size	3.764 (2.621–4.907)	< 0.001*	3.749 (2.664–4.835)	< 0.001*
Perithyroidal extension	1.692 (−1.153–4.537)	0.238		
Cervical lymph node metastasis	1.512 (−1.455–4.479)	0.311		
BRAF mutation	2.936 (−0.076–5.948)	0.056	2.859 (0.665–5.052)	0.012*

CI confidence interval

**p*<0.05 was considered significant

correction may reduce this problem regarding analysis of small sized tumors. However, as when we semiquantitatively measured the ¹⁸F-FDG uptake using harmonized SUV_{max}, both the tumor size and BRAF mutation were significantly associated with SUV_{max} in the larger size tumor patient subgroup (≥ 1 cm), further investigations regarding the relationship between tumor size and ¹⁸F-FDG uptake will be needed.

Recently, a similar study showing the correlation between BRAF mutation and glucose metabolism was reported by Nagarajah J et al. [17]. They showed that BRAF-mutant differentiated PTCs are significantly more ¹⁸F-FDG avid than BRAF wild-type tumors. In their study, ¹⁸F-FDG uptakes of local recurrence or metastatic PTC lesions on PET/CT images were analyzed, although BRAF mutation analyses were performed for primary tumors more than 1 year before PET/CT imaging, while we analyzed both the BRAF mutation status and the ¹⁸F-FDG uptake within a 6-month interval in the same primary PTC nodules, together with other clinicopathologic factors. Our study results showed more direct evidence for the correlation of BRAF mutation and glucose metabolism in PTCs, as the tumor differentiation and BRAF status might differ between the primary tumor and metastases.

Perithyroidal extension and cervical lymph nodes are well-known prognostic factors of PTCs and their relationship with

¹⁸F-FDG uptake has been reported by many researchers [2, 4, 5]. However, there were also conflicting reports [1–3, 6] that those parameters are not an independent predictor of ¹⁸F-FDG uptake in PTC, as shown in our study. These results might reflect that the high ¹⁸F-FDG uptake in cancer cells is not necessarily the consequence of rapid proliferation, but is caused by the activation of oncogenic pathways that regulate transporters and enzymes involved in the metabolism of glucose [18].

Several studies have suggested that an increased ¹⁸F-FDG uptake in BRAF mutant PTCs may be due to increased expression of GLUT-1 [9, 10, 13]. GLUT-1 expression is well correlated with ¹⁸F-FDG uptake in several cancers [19–22]. However, recent contradictory studies reported that ¹⁸F-FDG uptake in PTC was not correlated with GLUT-1 [1, 3, 23]. Rather than GLUT-1, ¹⁸F-FDG uptake of PTC may be associated with HIF-1 α [3] or GLUT-3 or -4 [1]. Zerilli et al. [24] suggested that the BRAF mutation-mediated signaling pathway may regulate the expression of HIF-1 α . Further studies are needed to clarify the relationship of ¹⁸F-FDG uptake, the expression of variable glucose transporters, HIF-1 α , and the BRAF mutation.

In our study, three patients with PTCs with relatively larger tumor sizes (1.0, 1.1, and 1.2 cm) showed non-¹⁸F-FDG

Table 4 Multivariate logistic and linear regression analysis for determining the factors associated with ¹⁸F-FDG avidity and SUV_{max} in the patient subgroups, according to the tumor size

Characteristics	Logistic regression analysis		Linear regression analysis	
	OR (95 % CI)	<i>p</i> value	B (95 % CI)	<i>p</i> value
Size (< 1 cm, <i>n</i> =26)				
Size	15.944 (0.064–3998.162)	0.326	1.361 (−0.608–3.329)	0.166
BRAF mutation	1.835 (0.333–37.432)	0.295	0.355 (−0.373–1.084)	0.323
Size (≥ 1 cm, <i>n</i> =29)				
Size	44.686 (0.758–2635.553)	0.068	3.950 (2.068–5.833)	< 0.001*
BRAF mutation	26.985 (1.660–438.692)	0.021*	5.323 (1.259–9.387)	0.012*

OR odds ratio, CI confidence interval

**p*<0.05 was considered significant

avidity, even though those tumors had the BRAF mutation. There were also three patients who had smaller PTCs (0.5, 0.6, and 0.8 cm) without the BRAF mutation, but with a high ^{18}F -FDG uptake. Although there is a possibility of false-negative or -positive results for BRAF mutation analysis, these patients suggest that factors other than tumor size or BRAF mutation status are associated with ^{18}F -FDG avidity, such as the differentiation status, RAS or RET/PTC oncogene, etc. [11, 12].

The clinical relevance of this study might be that the results could offer a suggestion regarding the usage of ^{18}F -FDG PET in PTC patients. The current American Thyroid Association guidelines limit the appropriate use of ^{18}F -FDG PET scanning to high-risk patients with non-iodine-avid tumors, Hurthle cell carcinoma, etc. [25]. According to this result, we might predict BRAF mutation status with high specificity using ^{18}F -FDG PET in tumors larger than 1 cm. In our study, all tumors with a $\text{SUV}_{\text{max}} > 5.2$ had BRAF mutation. This finding might have clinical significance in cases of thyroid incidentaloma seen on ^{18}F -FDG PET in which thyroid cancers are manifested as ^{18}F -FDG-avid nodules. Our study results might also be pertinent to therapeutic decision-making. There is growing evidence that BRAF mutation-targeted therapy (sorafenib) is effective in metastatic or advanced thyroid cancer [26–28]. There was also a report that in iodine-refractory differentiated thyroid cancer, an early ^{18}F -FDG PET response was useful for identifying sorafenib non-responders, although no BRAF mutation analysis was performed in those patients [29]. As for other targeted agents, such as gefitinib and erlotinib for lung cancer [30–33], ^{18}F -FDG PET/CT may have a role in BRAF mutant PTC treated with sorafenib.

There are several limitations to this study. First, it was a retrospective study with a relatively small number of patients. A selection bias could have been introduced as only a small proportion of the PTC patients who had thyroid surgery underwent both preoperative PET/CT and analysis for BRAF mutation within 6 months. The interval between PET/CT and the thyroid surgery was longer than 6 months in most of the patients who underwent ^{18}F -FDG PET/CT for other malignancy work-up. However, when we performed univariate and multivariate logistic regression analyses with staging work-up patients only, similar results to those of the total patients were obtained, although between the preoperative work-up and other malignancy work-up patients, patient age and lymph node metastasis status were significantly different. Therefore, the study results showing the association of the BRAF mutation and ^{18}F -FDG avidity may not have been heavily influenced by a selection bias. Second, not all patients underwent a total thyroidectomy and lymph node dissection, and about 70 % of patients were in a relatively early stage (stage I or II). Therefore, the clinicopathologic factors may not be properly evaluated. Third, because several different PET/CT scanners were used that differed in sensitivity and spatial resolution, it would have affected the results, as many

small tumors with < 1 cm were included in this study. This scanner difference might be mitigated by voxel resampling and SUV harmonization for proper quantification, although the spatial resolution and image quality were not optimal for the detection of ^{18}F -FDG avid lesion on each scanner and were more affected by the partial volume effect. Therefore, we generated two sets of images for each PET/CT examination, i.e., one to provide optimal lesion detection and the other to meet the quantitative harmonizing standards [34], after which both visual analysis of the ^{18}F -FDG avidity and semi-quantitative analysis of the SUV_{max} were performed. The visual analysis was advantageous for small lesion detection, and the kappa statistics showed that inter-observer agreement for analysis of the ^{18}F -FDG avidity was very good. To clarify the partial volume effect in small lesions, we also performed subgroup analysis according to the tumor size, i.e., < 1 and ≥ 1 cm. Therefore, despite these limitations, we believe that they had a very minor impact on the ability of our study to show an association between BRAF mutation status and the ^{18}F -FDG avidity in PTCs.

Conclusion

Among the clinicopathologic factors, the BRAF mutation is independently associated with the ^{18}F -FDG avidity of PTCs of ≥ 1 cm in size. The BRAF mutation must, therefore, be one of the most important factors influencing ^{18}F -FDG avidity in PTCs.

Conflict of Interest Suk Hyun Lee, Sangwon Han, Hyo Sang Lee, Sun Young Chae, Jong Jin Lee, Dong Eun Song, and Jin-Sook Ryu declare that they have no conflict of interest.

Ethical Statement This study was performed in accordance with the 1964 Helsinki Declaration and was approved by the Institutional Review Board of Asan Medical Center (IRB No. 2014–1002).

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