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Correlation of hypoxia inducible transcription factor in breast cancer and SUVmax of F-18 FDG PET/CT

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Abstract

BACKGROUND: Tumor hypoxia induces the expression of several genes via the hypoxia-inducible transcription factor-1 alpha (HIF-1α). It is associated with the prognosis of several cancers. We studied the immunohistochemical expression of HIF-1α in patients with invasive ductal cancer (IDC) of the breast and the possible correlation with the maximum standardized uptake value of the primary tumor (pSUVmax) as well as other biological parameters. Prognostic significance of pSUVmax and expression of HIF-1α for the prediction of progression-free survival (PFS) was also assessed.

MATERIAL AND METHODS: Two-hundred seven female patients with IDC who underwent pretreatment fluorine-18 fluorodeoxyglucose positron emission tomography/computed tomography (F-18 FDG PET/CT) were enrolled. The pSUVmax was compared with clinicopathological parameters including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), axillary lymph node (LN) metastasis, stage and HIF-1α expression. The prognostic value of pSUVmax for PFS was assessed using the Kaplan-Meier method.

RESULTS: pSUVmax was significantly higher in patients with HIF-1α expression $\geq$ 2 compared to patients with HIF-1α expression < 2 (5.2 ± 4.5 vs. 3.7 ± 3.1, p = 0.008). pSUVmax was also significantly higher in higher stage (p < 0.000001), ER-negative tumors (p < 0.0001), PR-negative tumors (p = 0.0011) and positive LN metastasis (p = 0.0013). pSUVmax was significantly higher in patients with progression compared to patients who were disease-free (6.8 ± 4.4 vs. 4.1 ± 3.7, p = 0.0005). A receiver-operating characteristic curve demonstrated a pSUVmax of 6.51 to be the optimal cutoff for predicting PFS (sensitivity: 53.6%, specificity: 86.0%). Patients with high pSUVmax (> 6.5) had significantly shorter PFS compared to patients with low pSUVmax (p < 0.0001).

CONCLUSIONS: pSUVmax on pretreatment F-18 FDG PET/ CT reflect expression of HIF-1α and can be used as a good surrogate marker for the prediction of progression in patients with IDC. The amount of FDG uptake is determined by the presence of glucose metabolism and hypoxia in breast cancer cell.

KEY words: HIF-1α, invasive ductal cancer of breast, F-18 FDG PET/CT, SUVmax
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Background

Breast cancer is the most frequent malignancy in women in the United States and the second most common cause of cancer-related mortality. Although it is curable when detected early, about one-third of women with breast cancer eventually die of the disease. Breast cancer is a remarkably heterogeneous disease. Therefore, precise prediction of prognosis and selection of optimal treatment are important [1].

Hypoxia is an important cellular stressor that triggers a survival program by which cells attempt to adapt to the new environment. This involves adaptation of metabolism and/or stimulation of oxygen delivery. These cell-rescuing mechanisms can be conducted rapidly by hypoxia-inducible factor-1 (HIF-1), a transcription factor that reacts to hypoxic conditions [2]. HIF-1 stimulates processes like angiogenesis, glycolysis and erythropoiesis [3] by activating genes that are responsible for these processes. The HIF-1 complex consists of two subunits, HIF-1α and HIF-1β. Protein concentrations of HIF-1α depend on the cellular oxygen concentration [4, 5].

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Cancer cells are able to survive and proliferate in extreme microenvironmental circumstances and show changes in oncogenes and tumor suppressor genes. Hypoxia and HIF-1 have been implicated in carcinogenesis and in clinical behavior of tumors. Up-regulation of HIF-1α is noted during breast carcinogenesis, especially in the poorly differentiated pathway [6]. Hypoxia is related to poor response to therapy in various cancer types. In invasive breast cancer, high HIF-1α concentrations have been associated with poor survival in lymph node (LN) negative patients [7]. As prognosis in breast cancer is closely related to proliferation rate and poorly differentiated tumors usually exhibit high proliferation and HIF-1α overexpression [8], the prognostic value of HIF-1α might well be explained by a close association between HIF-1α and proliferation.

Traditionally, pathological determination of the tumor size, histological tumor grade, axillary LN involvement, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status have driven prognostic predictions for patients with breast cancer. However, HIF-1α is not recognized as a prognostic factor for breast cancer.

F-18 fluorodeoxyglucose positron emission tomography/computerized tomography (PET/CT) is widely used in clinical practice for the diagnosis, staging, treatment monitoring and detection of disease recurrence in breast cancer patients [9]. PET/CT provides quantitative data on the level of metabolic activity by calculating the degree of F-18 FDG uptake, known as the standardized uptake value (SUV). PET/CT also has been suggested to have a considerable prognostic utility in various cancers [10–13]. In a previous study, primary tumor maximum SUV (pSUVmax) exhibited a strong relationship to known prognostic parameters of invasive ductal cancer (IDC) of the breast and could be used as a good surrogate marker for the prediction of progression in patients with IDC [14]. But the relationship between HIF-1α and pSUVmax was not clearly reported in IDC.

Therefore, we investigated the relationship between the maximum SUV of the primary tumor (pSUVmax), HIF-1α and known prognostic parameters of breast cancer. Prognostic value of pSUVmax was evaluated for the prognosis of progression-free survival (PFS) in patients with newly diagnosed primary IDC.

Materials and methods

Subjects

A total of two-hundred seven IDC female patients (age 25–90 years, mean age; 52.4 $\pm$ 9.5 years) who underwent F-18 FDG PET/CT before any cancer treatment from May 2005 to November 2010 were enrolled in this study. Primary breast cancer was diagnosed histopathologically with fine-needle aspiration cytology (FNAC) and/or core-needle biopsy (CNB). Patients with a history of insulin-dependent diabetes mellitus or who were diagnosed with excision biopsy were excluded. Primary tumor features included tumor size, ER, PR, HER2 status and HIF-1α status (Table 1). To obtain the tumor size, the longest diameter of the tumor was carefully measured from the ultrasonography (USG). Mammography, breast USG, magnetic resonance image, CT, whole body bone scan, and F-18 FDG PET/CT were used for the diagnosis of disease recurrence, metastasis, or progression, and all suspicious lesions were confirmed histologically by FNAC. Progression was defined as progression of disease to a more advanced TNM stage, a more advanced clinical stage, or relapse noted on images with histological confirmation.

F-18 FDG PET/CT

All patients fasted for at least 6 h before the administration of F-18 FDG, and blood glucose concentration was confirmed to be < 150 mg/dl in all subjects. Approximately 8.1 MBq of F-18 FDG per kg body weight was injected intravenously, and patients were advised to rest for an hour before acquisition of the PET/CT image. PET/CT scans were performed using a Discovery STE (GE Healthcare, Milwaukee, WI; 6-slice CT). First, a low-dose CT scan was obtained for attenuation correction, and the PET scan was followed at 3 min per bed position. PET data were reconstructed iteratively using an ordered-subset expectation maximization algorithm with the low-dose CT data sets for attenuation correction. An SUV was measured for all primary breast cancer lesions and presented as the SUVmax. The PET/CT images were interpreted by two experienced nuclear medicine physicians and a final consensus reached for all patients. A region of interest (ROI) was placed manually over all breast tumors in attenuation corrected images, and the SUVmax within the ROIs was recorded.

Construction of tissue microarrays (TMA)

Representative paraffin tumor blocks were selected according to the primary evaluation of hematoxylin and eosin (H & E) stained slides before they were prepared for TMA. Two tumor tissue cores (2 mm in diameter) were taken from each of the donor breast cancer tissue blocks with a Quick-Ray™ manual punch arrayer (Uni-Tech Science, Seoul, South Korea). The cores were placed in a new recipient paraffin block that ultimately contained 46 tissue cores. Totally 18 TMA blocks are used, each array block contained both tumor and control tissue samples. Multiple sections (5 μm thickness) were cut from the TMA blocks and then mounted onto microscope slides. The TMA H & E stained sections were reviewed under light microscopy to confirm the presence of representative tumor areas.

Immunohistochemical staining and interpretation

Immunohistochemistry was conducted on 5 μm-thick TMA tissue sections using the Bond Polymer Intense Detection System (Leica Microsystems, Nusslock, Germany) according to the manufacturer's instructions with minor modifications. Briefly, the 5 μm thick sections of formalin-fixed and paraffin-embedded TMA tissues were deparaffinized with Bond Dewax Solution (Leica Microsystems), and an antigen retrieval procedure was performed using Bond ER Solution (Leica Microsystems, Nusslock, Germany) for 30 min at 100°C. The endogenous peroxidase was quenched by 5-min incubation with hydrogen peroxide. Sections were incubated for 15 min at ambient temperature with commercially available primary monoclonal antibodies for ER (1:100, clone 6F11, Novocastra, Newcastle, United Kingdom), PR (1:100, clone 16, Novocastra), HER2 (1:250, A0485, Dako, Carpinteria, CA), and HIF-1α (1:50, clone H1alpha67, Novus Biologicals, Litten CO, USA) using a biotin-free polymeric horseradish peroxidase-linker antibody conjugate system in a Bond-Max automatic slide stainer (Leica Microsystems).

The expressions of hormonal receptors, ER and PR, were recorded according to the American Society of Clinical Oncol-

Table 1. Patients with IDC: pSUVmax values and recurrence according to prognostic factors

Variables	N (%)	pSUVmax (mean ± SD)	p value
Tumor size			
T1b; 0.5 cm < size ≤ 1.0 cm	47 (22.2)	2.6 ± 2.5	< 0.000001
T1c; 1.0 cm < size ≤ 2.0 cm	79 (38.1)	3.8 ± 3.6	
T2; 2.0 cm < size ≤ 5.0 cm	76 (36.7)	5.9 ± 3.9	
T3; 5.0 cm < size	5 (3.0)	8.2 ± 7.8	
Estrogen receptor (ER)			
Negative	63 (30.4)	6.8 ± 4.9	< 0.0001
Positive	144 (69.6)	3.4 ± 2.8	
Progesterone receptor (PR)			
Negative	56 (27.0)	6.2 ± 5.1	0.0011
Positive	151 (73.0)	3.8 ± 3.1	
HER2			
Negative	42 (20.2)	5.6 ± 5.2	0.1720
Positive	165 (79.8)	4.1 ± 3.4	
HIF-1α			
Negative (< 2)	110 (53.1)	3.7 ± 3.1	0.008
Positive (≥ 2)	97 (46.9)	5.2 ± 4.5	
Axillary LN involvement			
Negative	129 (62.3)	4.0 ± 4.1	0.0013
Positive	78 (37.7)	5.1 ± 3.4	
Stage, pathologic			
I	99 (47.8)	3.1 ± 3.4	< 0.000001
II	76 (36.7)	5.8 ± 4.1	
III	26 (12.6)	5.2 ± 3.4	
IV	6 (2.9)	6.2 ± 4.4	
Recurrence			
Recurrence-free	179 (86.4)	4.1 ± 3.7	0.0005
Recurrence	28 (13.6)	6.8 ± 4.4	

ogy/College of American Pathologists (ASCO/CAP) guidelines [15]. Cases with an HER-2 IHC staining score of > 2 were tested by HER2 gene amplification using the fluorescence in situ hybridization (FISH) method. HER-2 positive was defined as an IHC staining score of 3+ or, in the case of an IHC staining score of 2+, FISH positivity. The HIF-1α expression was scored according to the intensity of tumor cells exhibiting cytoplasmic staining (0 = none, 1 = weak, 2 = moderate, and 3 = strong). Moderate or strong cytoplasmic staining was considered as positive expression.

Statistical analyses

Numeric data are expressed as the mean ± SD. Moreover, to compare pSUVmax, HIF-1α expression was divided into two groups according to thresholds (2): negative (HIF-1α expression < 2) and positive (HIF-1α expression ≥ 2). The relationship between levels of primary tumor SUVmax (pSUVmax) and clinicopathological parameters was evaluated using the Mann-Whitney U-test and the Kruskal-Wallis test using MedCalc software, version 15.11.4 (MedCalc, Mariakerke, Belgium). To identify an optimal cut-off value of pSUVmax for the prediction of progression, receiver-operating characteristic (ROC) analysis was performed. Moreover, we used the log rank test according to the all patient's pSUVmax. Cutoff value was established by the maximum log rank statistical value. A pSUVmax higher than the cutoff value was defined as a "high SUVmax", and a pSUVmax below the cutoff value was defined as a "low SUVmax". Kaplan-Meier curves for high vs. low pSUVmax were calculated for PFS by logrank test. A P-value of < 0.05 was considered to be statistically significant.

Results

Patient characteristics

Pertinent characteristics of the patients are summarized in Table 1. The mean age was 52.4 ± 9.5 years, ranging from 25 to 90. The median follow-up period was 49 months (mean ± SD = 48.8 ± 20.7, range: 26–97 months). Tumor stage was categorized by the size of primary tumor (T): 126 (60.3%) in T stage 1, 76 (36.7%) in T stage 2 and 5 (3.0%) patients in T stage 3. Axillary LN involvement at pathology was observed in 78 patients (37.7%). There were 99 (47.8%) patients in stage I, 76 (36.7%) in stage II, 26 (12.6%) in stage III, and 6 (2.9%) in stage IV IDC (Table 1). All 207 patients underwent surgery. While 151 patients received chemotherapy, 111 received radiotherapy, and 151 received hormonal therapy, according to

clinical status. 179 (86.4%) patients were in a disease-free status, and 28 (13.6%) patients presented a progression during follow-up.

Relationship between pSUVmax and clinicopathological parameters

Table 1 shows pSUVmax differences according to the clinicopathological parameters. Mean pSUVmax of the 207 patients was 4.4 ± 3.9 (range, 0.5 to 21.3). The mean pSUVmax was significantly different among the T-stage groups ($p < 0.000001$) and was increased as tumor size increased. pSUVmax was significantly higher in ER-negative tumors ($p < 0.0001$), PR-negative tumors ($p = 0.0011$), HIF-1α positive expression ($p = 0.008$) and tumors with positive LN metastasis ($p = 0.0013$) compared to ER-positive tumors, PR-positive tumors, HIF-1α negative expression and tumors without LN metastasis, respectively. However, there was no significant difference in pSUVmax according to HER2 status ($p = 0.1720$). The mean pSUVmax was 3.1 ± 3.4 in stage I, 5.8 ± 4.1 in stage II, 5.2 ± 3.4 in stage III, and 6.2 ± 4.4 in stage IV, respectively, which were significantly different between each group ($p < 0.000001$).

PFS

Twenty eight (13.6%) of the 207 patients experienced progression, and the median follow-up time to progression was 49 months (mean ± SD = 48.8 ± 20.7, range: 26–97 months). pSUVmax was significantly higher in patients with progression than in those who were disease-free. The mean pSUVmax of the disease-free group was 4.1 ± 3.7 and that of the progression group was 6.8 ± 4.4 ($p = 0.0005$, Figure 1, 4 and 5).

A ROC curve demonstrated a pSUVmax of 6.8 to be the optimal cutoff for predicting PFS (area under the curve: 0.727; standard error: 0.0471) (Figure 2). A pSUVmax of 6.8 yielded a sensitivity of 53.6% and a specificity of 86.0% for prediction the PFS. Also, the result of the log-rank test according to the all patient's pSUVmax showed a cutoff value of 6.6, representing maximum log rank statistical value ($p = 0.0001$).

In the survival analysis using the Kaplan-Meier method, patients with a pSUVmax > 6.8 had a significantly shorter PFS than patients with pSUVmax < 6.8 ($p < 0.0001$, Figure 3–5).

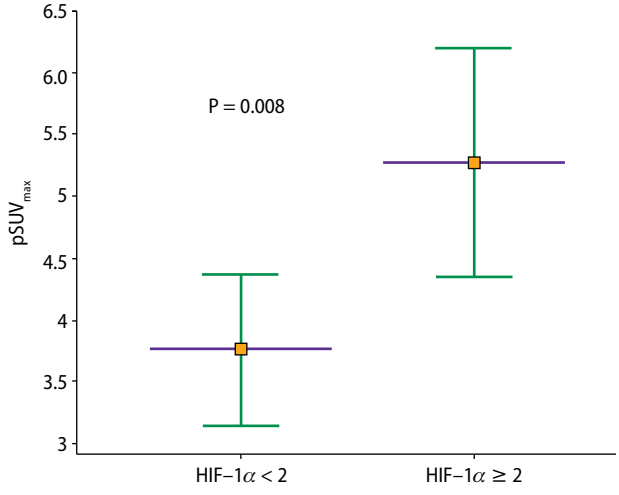

Figure 1. pSUVmax differences according to HIF-1α expression group. Mann-Whitney U-test reveals a significant difference between the HIF-1α negative (< 2) group and HIF-1α positive (≥ 2) ($p = 0.008$). Mean values of pSUVmax (3.73 in the HIF-1α negative and 5.16 in the HIF-1α positive) are indicated with red boxes. The error bars represent the 95% confidence interval for mean

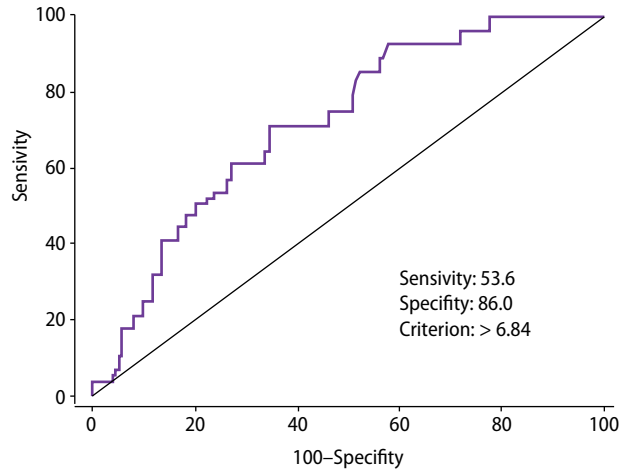

Figure 2. Optimal cutoff of pSUVmax for predicting progression-free survival

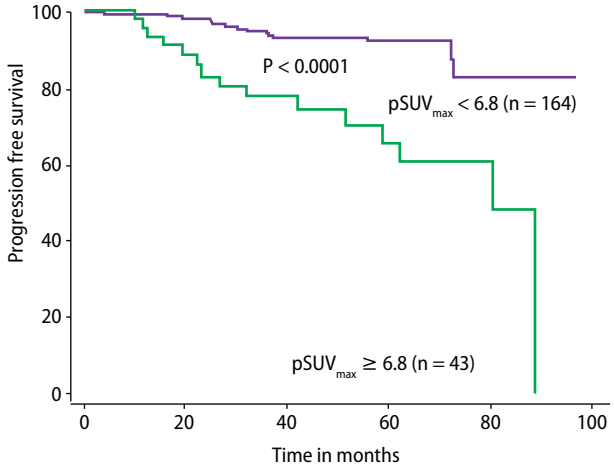

Figure 3. Progression-free survival according to the pSUVmax

Discussion

To our knowledge, this is the first retrospective study to evaluate the biologic correlation of pSUVmax and expression of HIF-1α in patients with IDC. Cells subjected to hypoxia must undergo metabolic adaptations to survive. Under hypoxic conditions there is also a parallel increase in glucose uptake, which is facilitated by up-regulation of Glut1 expression. Hypoxia is a potent stimulus for Glut1 mRNA induction in a variety of tumor cell lines [16]. The mechanism of Glut1message induction by hypoxia is complicated and is dually controlled by low oxygen concentration and by inhibition of oxidative phosphorylation [17]. The cobalt-responsive element in the rat Glut1 promoter has been mapped and is homologous to the mouse Enhancer-1 sequence [16, 17]. Transactivation through this element in the promoter is mediated by HIF-1 [16] that binds to

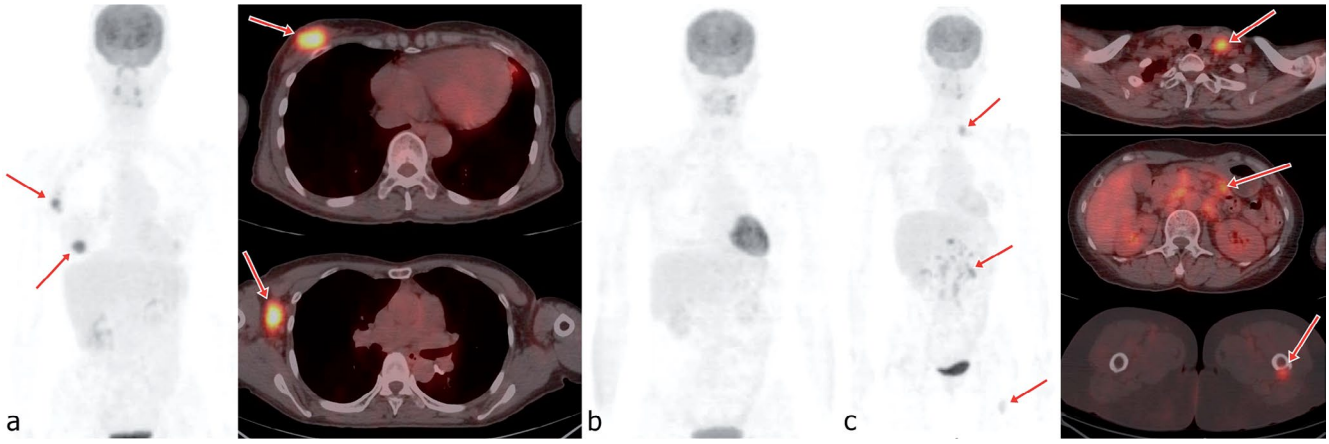

Figure 4. A. A 57-year-old female patient diagnosed with IDC (pSUVmax 10.0, tumor size 3.5 cm, ER-, PR+, HER2+, HIF-1α 3+) and underwent PET/CT before cancer treatment. In the pre-treatment, a focal hypermetabolic lesion in the right breast (red arrow) and enlarged LNs with focal FDG uptake in the right axillary area (SUVmax 10.7, red arrow) were shown, which were histologically confirmed as malignancy in both lesions (long arrow); **B.** In the follow-up PET/CT taken after neoadjuvant chemotherapy and operation, there was no evidence of residual malignancy; **C.** But 14 months later, newly found hypermetabolic lesions in the left supraclavicular (SUVmax 6.3, red arrow), abdominal LNs (8.2, red arrow) and left femur (6.3, red arrow) on the follow-up F-18 FDG PET/CT was shown and radiological and histological confirmed as metastasis

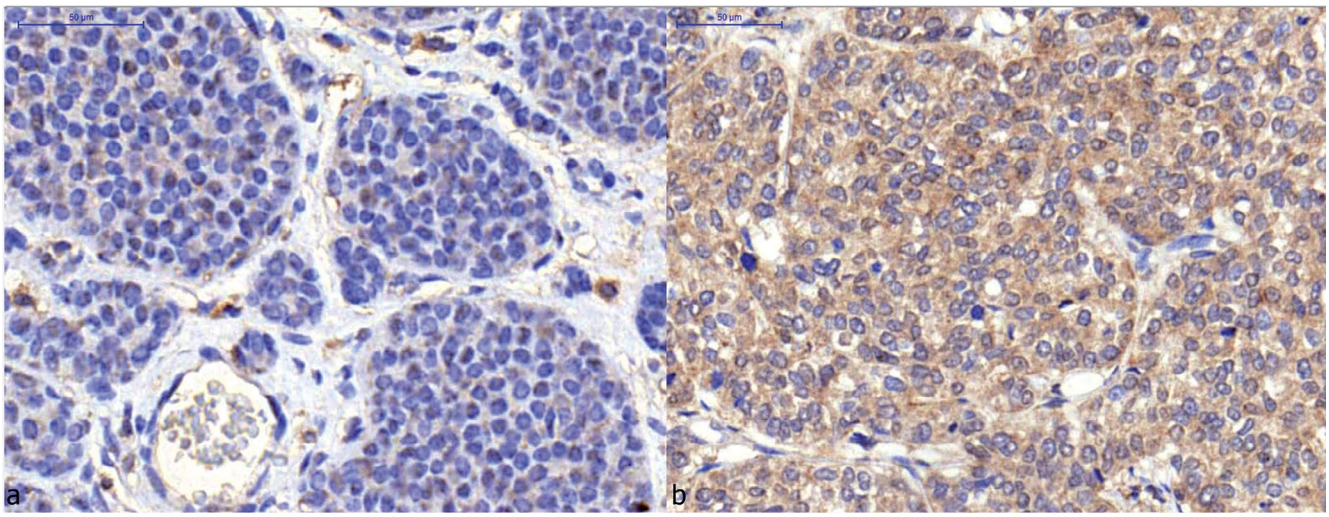

Figure 5. A. A 68-year-old female patient diagnosed with invasive IDC (pSUVmax 1.0, tumor size 1.5 cm, ER+, PR+, HER2+, HIF-1α 1+) and no recurrence of IDC during 5-year follow-up period; **B.** A 43-year-old female patient diagnosed with IDC (pSUVmax 12.6, tumor size 3.0 cm, ER-, PR-, HER2-, HIF-1α 3+) who developed bone metastases and died after 2 years

a specific DNA consensus sequence, 59-RCGTG-39, found in the promoters of many hypoxia-inducible genes [18]. The HIF-1 transcription factor consists of two subunits, HIF-1α and HIF-1β. HIF-1α protein, which is unique to the HIF-1 complex, is rapidly degraded in oxygenated cells by the ubiquitin-proteasome pathway [19]. HIF-1α protein is specifically induced by hypoxia in a graded response dependent on the oxygen concentration [5].

Determination of malignant lesions with PET/CT is based on their glucose metabolism [20, 21]. The overexpression of Glut1 is closely related to FDG uptake in human cancer [20, 21]. Glut1 is thought to be a possible intrinsic marker of hypoxia, and its expression is regulated by hypoxia via HIF-1 [22, 23]. Hypoxic conditions may correspond to higher FDG uptake [24]. In addition, several studies described the relationship between FDG uptake and the expression of vascular endothelial growth factor (VEGF) or micro-vessel density (MVD) [25, 26]. HIF is considered to support tumor growth by the induction of angiogenesis via the expression of VEGF and also by anaerobic metabolic mechanisms [27]. In this study, we found that high pSUVmax was related to poor outcome in patient with IDC and indicated that high HIF-1α expression was also related to poor prognosis.

Various prognostic factors have been proposed for the risk stratification of patients with breast cancer, such as involvement of axillary LNs, presence of distant metastases, hormonal receptor status and HER2 status. However, these pathological predictors can only be obtained after surgery, which is frequently associated with significant morbidity and mortality. Also tissue samples sometimes cannot be obtained even with invasive diagnostic procedures. On the other hand, PET/CT can provide quantitative information about tumor glucose metabolism, which represents the aggres-

siveness of the malignant lesion. FDG uptake can be evaluated noninvasively and be measured with good inter-test reproducibility [28]. Therefore, quantitative FDG uptake can be a valuable adjunct to conventional preoperative clinical assessment. In our study, a statistically significant difference in recurrence-free survival was also observed between patients with high pSUVmax and those with low pSUVmax.

Our study had limitations. First, fine needle aspiration and/or core-needle biopsy were performed to diagnose IDC in all patients. Consequently, the pSUVmax might have been affected. It is possible that inflammation after a procedure could have contributed to the pSUVmax, although this may not have been significant. Considering the design of the study, this type of effect was inevitable and did not adjust the results described. Second, there was wide overlap of pSUVmax between patients with and without recurrence. Prediction of recurrence would not be easy in patients with borderline value of pSUVmax.

Conclusion

pSUVmax has a strong relationship to expression of HIF-1α and known prognostic parameters of breast cancer and could be useful to predict the prognosis in patients with IDC. The relationship between pSUVmax and HIF-1α may lead to a more rational use of PET/CT in patients with IDC.

Compliance with Ethical Standards

Ethical approval

The original article was approved by an institutional review board (IRB No. CR-15-052-L). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the principles of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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Conflict of interest

All authors declare that there is no conflict of interest.

Authors' contributions

SMK participated in design of manuscript and provide medical writing and drafted the manuscript. YJJ, YYC and SHP participated in design of manuscript and coordination and helped to draft the manuscript. JWJ and HKO participated in analysis of pathological specimen. All authors read and approved the final manuscript.

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