TIGAR Is Correlated with Maximal Standardized Uptake Value on FDG-PET and Survival in Non-Small Cell Lung Cancer

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Abstract

Objective: Evaluation of 18F-FDG uptake value via PET is central to current methods of diagnosis and staging of non-small cell lung cancer (NSCLC) due to its ability to evaluate expression levels of key regulators associated with glucose metabolism in tumor cells. Tp53-induced glycolysis and apoptosis regulator (TIGAR) is an important P53-induced protein that can inhibit glycolysis; however, there have been few clinical studies on its mechanism. Here we have investigated the relationship between TIGAR expression and 18F-FDG PET in tumors, along with its relationship with the clinical characteristics of NSCLC.

Methods: We analyzed SUVmax in 79 patients with NSCLC through immunohistochemical staining of TIGAR and five other biological markers associated with tumor cell glycolysis, in order to evaluate the correlation between their expression and SUVmax. We also plotted Kaplan-Meier survival curves to assess TIGAR expression with the prognosis and survival of patients with NSCLC.

Results: The key findings were as follows: SUVmax was negatively correlated with the expression of TIGAR (r = −0.31, p < 0.01); TIGAR expression was correlated with tumor size (p = 0.01), histological type (p < 0.01), differentiation degree (p < 0.01), and lymph node metastasis (p < 0.01) in patients with NSCLC; and the survival time of patients whose TIGAR was negatively expressed was significantly shorter than for those whose TIGAR was positively expressed (P = 0.023).

Conclusions: The expression of TIGAR in primary tumors is significantly correlated with SUVmax and low expression of TIGAR may predict a worse clinical outcome in patients with NSCLC.

Citation: Zhou X, Xie W, Li Q, Zhang Y, Zhang J, et al. (2013) TIGAR Is Correlated with Maximal Standardized Uptake Value on FDG-PET and Survival in Non-Small Cell Lung Cancer. PLoS ONE 8(12): e80576. doi:10.1371/journal.pone.0080576

Received August 22, 2013; Accepted October 14, 2013; Published December 10, 2013

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Unlike normal cells, most cancer cells depend on a high rate of glycolysis for energy production during malignant progression. This is known as the Warburg effect and is considered the seventh hallmark of cancer [1]. This effect has been exploited in 18F-fluorodeoxyglucose positron emission tomography/computerized tomography (18F-FDG PET/CT) technologies, which has proved highly successful in clinical practice and is widely applied in tumor diagnosis [2,3].

The maximal standardized uptake value (SUVmax) determined through PET imaging is a simple and reliable method of evaluating the glucose uptake capacity of tumors in vivo. It is defined as the ratio of activity in tissue per unit volume to the activity in the injected dose per patient body weight [4]. Recent reports have demonstrated that the SUVmax of primary tumors is correlated with the stage, nodal status, histologic type, differentiation and progression of tumors [5,6]. Furthermore, a high SUVmax has been linked with poor prognosis in cancer patients [7].

Despite these observations, further investigations are required to establish the clinical value of 18F-FDG PET/CT imaging for tumor diagnosis, by identifying the metabolic enzymes and abnormal expressions of cancer genes that underlie SUVmax changes in cancer tissues. Therefore, many studies have focused on defining the relationship between FDG uptake and the expression of tumor biomarkers, including nuclear-associated antigen Ki-67, cyclooxygenase-2 (Cox-2), vascular endothelial growth factor (VEGF) and the glucose transporter 1 (GLUT1) in relation to lung cancer [4,8,9]. To date, these have reported a correlation between SUVmax and tumor biomarkers and have contributed to
understanding the Warburg effect and identifying tumor-related genes that are associated with the SUV, thereby providing a robust basis for staging, prognosis and personalized treatment of cancers.

TIGAR is a novel gene related to the glucose metabolism in tumor cells [10]. TIGAR inhibits glycolysis by limiting the level of fructose-2, 6-bisphosphatase (Fru-2, 6-BP) in the cell by functioning as a Fru-2, 6-bisphosphatase (Fru-2, 6-BPase). It was commonly believed that induction of TIGAR led to glycolysis inhibition in the cancer suppressor, P53 [11]. More recent studies have suggested that TIGAR expression increases NADPH levels through activation of the pentose phosphate pathway (PPP), thereby promoting antioxidant function which reduces reactive oxygen species (ROS)-associated apoptosis and enhances cancer cell survival [12]. However, these studies were conducted on a limited number of cell lines through intervening transient expression experiments; therefore, it remains to be established whether TIGAR expression in clinical practice, by acting as a suppressor gene of glucose metabolism.

The purpose of this present study was to assess the correlation between SUV\textsubscript{max} and the expression of selected markers associated with tumor metabolism, namely, glucose transporter 1 (GLUT1), hexokinase 2 (HK2), pyruvate kinase M2 (PKM2), lactate dehydrogenase A (LDHA), protein kinase B (AKT) and TIGAR. In addition, the relationship between the TIGAR expression and prognosis in non-small cell lung carcinoma (NSCLC) was investigated. We selected 79 NSCLC cases for this study, with the aim of identifying novel biological indicators for clinical diagnosis and personalized treatment options, by analyzing the relationship between SUV and key regulatory factors in glucose metabolism.

**Patients and Methods**

**Ethics Statement**

The Human Investigation Ethical Committee of Shanghai Jiao Tong University affiliated Renji Hospital and Shanghai Chest Hospital approved this study. All procedures involving human specimens were performed with written informed consent according to the Declaration of Helsinki.

**Study Population**

This was a retrospective study of all patients who were confirmed to have NSCLC based on histopathological finding and underwent surgery after \(^{18}\)F-FDG PET/CT between December 2006 and December 2009 at Shanghai Jiaotong University affiliated Renji Hospital and Shanghai Chest Hospital. Eligibility criteria were (1) without receiving chemotherapy/ radiotherapy before PET/CT scanning; (2) tumor pathology of NSCLC (excluded benign lung lesions and small cell lung cancers); (3) complete case records; (4) performed PET/CT scanning no more than 2 weeks prior to surgery; (5) available tissue specimen for IHC staining. Finally, 79 patients (50 male and 29 female) with a median age of 61 (range, 30–79 years) were evaluated in this study. All clinical and pathological findings were reviewed from three hospitals in Shanghai, China (Renji Hospital, Shanghai Chest Hospital, Ruijin Hospital).

**PET/CT imaging**

A dedicated whole-body PET/CT tomography (SIMENS) was used for all PET/CT imaging. Image acquisition was performed with an integrated PET/CT device. Immediately after CT scanning, PET was performed to cover the identical axial field of view. PET-image data sets were reconstructed iteratively with segmented correction for attenuation using the CT data. For semi-quantitative analysis of the \(^{18}\)F-FDG uptake, irregular regions of interest (ROIs) were placed over the most intense area of 18F-FDG accumulation. The maximum SUV (SUV\textsubscript{max}) was calculated using following formula: maximum pixel value with the ROI activity (MBq/kg/injected dose [MBq]/body weight [kg]). Two experienced nuclear medicine physicians, blinded to the clinical history, independently evaluated the PET images and reached a consensus on all image results.

**Immunohistochemical Staining**

Immunohistochemical analyses were performed on paraffin-embedded lung cancer tissues. After microtome sectioning (4 \(\mu\)m slices), the slides were processed for staining. All primary antibodies (TIGAR, GLUT-1, HK-2, PKM-2, LDHA, AKT) were purchased from Abcam. Sections were assessed using a light microscope (BX51TR, Olympus, Japan) by 2 independent observers. The expression of each marker protein was examined and statistical software (DFC320 CCD system, Leica, Germany) was used for semi-quantitative analysis of IHC staining. The slides were scored for intensity of staining (0 to 3) and the percentages of cells with scores of 0 (0%), 1 (% to 9%), 2 (10% to 49%), and 3 (50% to 100%) were determined. The immunohistochemistry (IHC) score (0 to 9) was defined as the product of the intensity and percentage of cells. Protein expression was judged as positive when the IHC score was greater than or equal to 4. All IHC results were evaluated by 2 experienced observers who were blind to the
condition of the patients. Where discrepancies occurred by 2 readers, the 2 readers reached a consensus.

Statistical Analysis
Continuous variables were analyzed by the Student’s t test, and the results were expressed as mean ± standard deviation. Dichotomous variables were analyzed by the χ2 test. We used Spearman rank correlation to examine the association between SUVmax and protein expression. To explore the association between recurrence-free survival and TIGAR expression, a Kaplan-Meier survival analysis was performed. Two-sided p values of less than 0.05 were considered to be statistically significant. All analyses were performed using SPSS software, version 16.0 (SPSS Inc, Chicago, IL).

Results
Clinical and pathological characteristics of patients in relation to SUVmax
The clinical and pathological characteristics of the 79 patients with NSCLC in this study are illustrated in Table 1. The patients were aged between 30 and 79 years (mean; 60.85 ± 10.40 years) and included 50 males and 29 females. The mean SUVmax was 7.24 ± 4.58, the maximum, minimum and median values were 24.1, 0.7 and 6.6 respectively, and 10 patients had a SUVmax ≤ 2.5. Statistical analysis showed that there were no significant differences in SUVmax between patients ≥ 60 years old and patients < 60 years old or between male and females patients. However, there were significant differences in SUVmax between patients with tumors > 3 cm and those with tumors ≤ 3 cm in size. In addition, SUVmax was statistically different between patients with well, moderate and poorly differentiated tumors; between patients with squamous carcinoma and adenocarcinoma and between patients with pathological stage N0 and N1 tumors (p < 0.01).

| Table 2. Pearson correlation coefficients and p-value between the immunohistochemistry (IHC) staining scores for genes expression associated with glucose metabolism in tumors with SUVmax. |
|---|---|---|
| Factor | correlation coefficients | SUVmax | P-value |
| TIGAR expression | 0.31 | <0.01 |
| GLUT-1 expression | 0.37 | <0.01 |
| HK-2 expression | 0.08 | 0.45 |
| PKM-2 expression | 0.21 | 0.06 |
| LDHA expression | 0.09 | 0.39 |
| AKT expression | 0.04 | 0.71 |

| Table 3. Correlation between TIGAR or GLUT-1 expression and SUVmax. |
|---|---|---|
| Factor | No. patients | SUVmax | P-value |
| TIGAR expression | | | <0.01 |
| Negative | 37 | 8.29 ± 3.64 |
| Positive | 42 | 6.31 ± 5.14 |
| GLUT-1 expression | | | <0.01 |
| Negative | 46 | 6.09 ± 3.60 |
| Positive | 33 | 8.83 ± 5.33 |
Relationship between FDG-PET and immunohistochemical scores of proteins involved in glucose metabolism in tumor cells

We carried out immunohistochemical (IHC) analysis in order to assess the correlation between PET SUV<sub>max</sub> and the expression of selected proteins associated with glucose metabolism in tumor cells according to their IHC staining scores.

The results are shown in Figure 1 and presented in Table 2. SUV<sub>max</sub> of FDG showed a negative correlation with TIGAR expression (r = -0.31, p<0.01). In addition, SUV<sub>max</sub> was significantly correlated with expression levels of GLUT1 (r = 0.37, p<0.01). However, SUV<sub>max</sub> was weakly correlated with PKM2 and not correlated with glucose metabolism genes, LDHA, HK2 or oncogenes, AKT.

There was a significant difference in SUV<sub>max</sub> between patients with positive and negative expression of TIGAR (P<0.01). The mean value of SUV<sub>max</sub> was 8.29±3.64 in the TIGAR negative cases and 6.31±3.14 in the TIGAR positive cases. Similarly, SUV<sub>max</sub> was significantly different between patients with positive and negative expression of GLUT1 (P<0.01). The mean value of SUV<sub>max</sub> was 6.09±3.60 in the GLUT1 negative cases and 8.83±5.33 in the GLUT1 positive cases (Table 3).

We further analyzed the impacts of the different expression level of TIGAR and GLUT1 on glucose uptake (Table 4). The maximum SUV<sub>max</sub> (mean, 10.20±3.40) was observed in patients with negative expression of TIGAR and positive expression on GLUT1. Conversely, the minimum SUV<sub>max</sub> (mean, 5.47±3.89) was observed in patients with positive expression of TIGAR and negative expression of GLUT1.

Relationship between TIGAR expression and the biological characteristics and staging grade of NSCLC tumors

Although TIGAR is closely linked to glucose uptake in tumor tissues, there have been few reports on its relationship with the biological characteristics of tumor tissues. Therefore we analyzed TIGAR expression levels in tissue samples from 79 patients with NSCLC in relation to age, gender, tumor size, histological type, histological degree and tumor staging. The results are presented in Table 5. These showed that the difference between TIGAR expression in respect of patient gender or age (<60 or ≥60 years) was not significant. Conversely, the difference was significant between TIGAR expression in patients with respect to tumor size (≤3 or >3 cm), histological type (squamous or adenocarcinoma), differentiation degree (well or poor) and staging (N0 or N1).
inducible factor 1 (HIF-1) increases [14–16]. However, the mechanisms underlying the Warburg effect are not yet fully understood.

In our present study, the expression levels of seven metabolic enzymes associated with glucose metabolism in tumor cells were compared with SUV_{max} in 79 patients with NSCLC. Our results showed that SUV_{max} of FDG uptake was significantly correlated with expression levels of GLUT1 and TIGAR, weakly correlated with PKM2 and not correlated with glucose metabolism genes, LDHA, HK2 or oncogenes, AKT.

Previous reports have suggested that GLUT1 has an important role in 18F-FDG uptake in patients with lung cancer, soft tissue sarcoma and pancreatic cancer [9,17,18]. Conversely, Tohma et al. reported low correlation between 18F-FDG uptake and GLUT1 expression in esophageal cancer [19]. Our data demonstrated that GLUT1 expression was significantly associated with high SUV_{max}, supporting earlier reports that GLUT1 expression contributes to 18F-FDG uptake in NSCLC patients.

Notably, this study has provided the first evidence of a correlation between TIGAR expression and SUV_{max} on FDG-PET in patients with NSCLC. Our data showed that TIGAR expression is negatively correlated with tumor SUV_{max}. Furthermore, SUV_{max} was highest in patients who had positive expression of GLUT1 with negative expression of TIGAR. This further demonstrated that TIGAR involved in glycolysis in lung cancer and TIGAR inhibition could remove the negative regulation of glucose metabolism, thereby increasing glycolysis in tumor cells.

Many experiments that have been performed at the cellular level have confirmed that TIGAR can inhibit glycolysis. This is consistent with our clinical observations. It is generally considered that the glycolysis mechanism induced by TIGAR bears close similarity to that of the bisphosphatase (FBPase) domain of 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase (PFK-2/ FBPase-2), in that TIGAR expression leads to a reduction in fructose-2, 6-bisphosphate (Fru-2, 6-BP) levels, thereby suppressing glycolytic flux and tumor growth. Fru-2, 6-BP is a potent allosteric regulator of PFK1, an early enzyme in the glycolytic pathway. Furthermore, the decrease in Fru-2, 6-BP levels may lead to decreased GK (the hepatic isoform of hexokinase) and increased G6Pase levels [20], providing an additional means by which TIGAR can negatively regulate glycolysis.

Activation of glycolysis is not only beneficial to tumor metabolism for energy production and proliferation, but can also activate multiple transcription factors associated with oncogenes and tumor development, such as sterol regulatory element binding protein (SREBP) which is involved in fatty acid synthesis [21]. Therefore, these glycolytic genes have also been the focus of studies on tumor development. It is increasingly apparent that TIGAR may play other significant roles in controlling processes involved in human malignancies; however, the mechanisms remain to be fully understood. It has been suggested that TIGAR inhibits tumor growth by acting in a similar manner to the tumor suppressor P53. López-Guerra et al. found that high expression of TIGAR was positively correlated with chronic lymphocytic leukemia (CLL) sensitivity to Fludarabine, which can induce apoptosis [22]. Madan et al. showed that TIGAR could stabilize the retinoblastoma protein and E2F transcription factor 1 (Rb-E2F1) complex leading to cell cycle arrest and inhibition of tumor growth [23]. Similarly, Hesegawa found that high expression of TIGAR promoted cell cycle arrest and cellular senescence, whereas a TIGAR knockout acted to reduce apoptosis [24]. However, a conflicting mechanism proposed by Bensaad et al. suggested that TIGAR inhibited glycolysis at the cellular level while activating the PPP, which inhibited ROS production and tumor cell apoptosis and autophagy; thereby promoting tumor growth in U2OS and RKO cell lines [12].
The majority of studies on TIGAR have been performed at the cellular level with only a few reports on clinical studies. Therefore, we conducted this study to compare TIGAR expression with only a few reports on clinical studies. Therefore, we conducted this study to compare TIGAR expression with only a few reports on clinical studies. We demonstrated that TIGAR expression was significantly higher in lung adenocarcinoma tissue than in squamous carcinoma (P<0.01), which is consistent with observations of lower SUV in lung adenocarcinoma. We found that well-differentiated NSCLC had higher expression levels of TIGAR compared to poorly differentiated NSCLC, indicating increased malignancy. In addition, we showed that patients with negative expression of TIGAR were more likely to develop lymph node metastasis than those with positive expression of TIGAR. These indicated that TIGAR expression was closely correlated with prognosis. Survival curve analysis showed that the survival rate declined significantly in NSCLC patients who had low levels of TIGAR expression (P=0.023) compared to those with high levels. These findings, based on clinical studies of NSCLC tissues, suggest that quantification of TIGAR protein levels may help predict prognosis of patients with NSCLC and cancer treatment targeting TIGAR might become feasible in the future. We have proposed to conduct a trial of a TIGAR activator for the treatment of lung cancer in PET-positive mice with the aim of developing potential gene targeted therapies for lung cancer.

In conclusion, the expression of GLUT1 and TIGAR was correlated to SUVmax in NSCLC. This is the first report that describes a significant correlation between the expression of TIGAR and SUVmax and showed that low expression of TIGAR in primary NSCLC tumors was strongly correlated with a worse clinical outcome. These findings indicate that TIGAR may help predict prognosis of cancer patients and facilitate selection of patients for targeted therapies involving TIGAR in NSCLC. Further studies are required to identify lung cancer patients who are suitable for TIGAR-targeted therapy based on pre-evaluation of FDG uptake.

Author Contributions
Conceived and designed the experiments: GH JJL. Performed the experiments: XZ WHX QL JZ. Analyzed the data: XZ WHX YFZ XPF. Contributed reagents/materials/analysis tools: XZ QL. Wrote the paper: XZ WHX.

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