The ^{18}F-FDG uptake in non small cell lung carcinoma correlates with the DNA-grading of malignancy

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Abstract In order to evaluate correlation of glucose metabolism and DNA ploidity of tumors, the uptake of ^{18}F-Deoxyglucose (FDG) by PET prior to surgery and the DNA cotent and DNA-grading of malignancy (DNA-MG) of Schiff-stained nuclei obtained from fresh tumor fragments by means of image cytometry were studied, and thereafter the correlation between standardized uptake value (SUV) and (DNA-MG) was analysed in forty-nine patients with histologically proven non-small cell lung carcinoma (NSCLC). As a result of the DNA histograms of these 49 patients, 46 (93.88%) were aneuploid and only 3(6.12%) were tetraploid. A linear correlation of the SUV versus the (DNA-MG) (r=0.336, p=0.024) was found, demonstrating that ^{18}F-FDG PET as a non-invasive metabolic imaging technique, may also provide information correlated to malignant DNA patterns which may be valuable in malignant differentiation and prognostic prediction.

Keywords Non small cell lung carcinoma, ^{18}F-FDG, Positron emission tomography, DNA-image cytometry, DNA-grading

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1 INTRODUCTION

The DNA distribution pattern in the tumor cell has been reported to be correlated with tumor malignancy, clinical stage, survival or tendency for metastatic spread.[1-3] On the other side, the FDG uptake, which could be assessed by a noninvasive FDG imaging in the routine clinical practice, might be correlated with histologic malignancy grading in brain tumors.[4] Therefore, we evaluated the relation between the regional glucose uptake and the DNA ploidity in a large number of patients with non small cell lung carcinoma (NSCLC).

2 MATERIALS AND METHODS

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2.1 Clinical material

We examined 49 patients (38 men, 11 women; age range 48 - 81; mean age 67.22±7.06) with NSCLC who had undergone ^{18}F-FDG PET prior to surgery. All patients had undergone surgery and the pathological diagnoses had been determined as followes: 15 patients had adeno-carcinoma, 31 had squamous carcinoma and 3 had adeno-squamous carcinoma.

2.2 PET procedure

Whole body FDG PET study from mid-thigh to base of skull (4–6 bed positions of 10 to 15 min each) was performed with ECAT EXACT (47 image planes of 3.4 mm; Siemens/CTI) 45–90 min after injection of 6 MBq/kg body weight (350–600 MBq) of ^{18}F-FDG which was produced by the radiopharmaceutical laboratory in Bad Berka, and the images were iteratively reconstructed as 3D images of coronal-, sagital- and trans-axials. The patients were fasted for 12–16 hours before PET scanning, hydrated (0.75–1 liter of mineral water, beginning approximately 15 min before the FDG injection) and intravenously administration of a diuretic (furosemide: 20–40 mg) 20 min after FDG injection. Plasma levels of glucose were measured at the time of FDG injection. Transmission images were obtained for 5–10 min per bed position in the form of a "hot transmission" scan to correct for photon attenuation.

Both attenuation-corrected and non-attenuation-corrected images were interpreted visually by two expert nuclear medicine physicians. The attenuation-corrected transaxial section expressing maximum FDG uptake by the primary tumor was analyzed semiquantitatively, using the standardized uptake value (SUV) which was calculated as: SUV =(activity in ROI in 37 MBq/L)/(injected dose in 37 MBq/weight in kg). The metabolic diameter of tumor (MTD) was determined based on the FDG uptake by the tumor on transaxial slices with the thickness of each slice of 5 mm.

2.3 DNA image cytometry

2.3.1 Preparation of smears

For DNA measurement, smears were prepared by scraping cells from fresh tumor fragments immediately after resection. Smears were fixed in methanol for 20 min, and then hydrolyzed in hydrochloric acid at room temperature for 35 min, stained with Schiff reagent at room temperature for 60 min.

2.3.2 Image cytometry

DNA measures were performed by means of an OPTIMAS-based analysis system equipped with an JENALUMAR microscope. Image cytometry was performed by an object-dependent segmentation procedure after interactive selection of the object on the screen. After measuring up to 300 tumor cells and 30 granulocytes (as reference), a DNA-histogram including diagnosis, $2cDI$, grading of malignancy (DNA-MG), etc. was

printed. The whole precedure takes about 30 min, and the most time-consuming part was the manual selection of cells.

Grading of Malignancy(3). From the single-cell DNA values the 2*c* deviation index($2cDI$) was computed as follows: $2cDI$ is defined as the sum of squares of the differences between the DNA values of the measured single cells and the 2*c* value, divided by the number of measured cells:

$$2cDI = N^{-1}\sum_{i=1}^{n}(c_i - 2c)^2$$

$2cDI$ represents the mean square deviation from the diploid value. The (DNA-MG) is computed from the $2cDI$ performing a logarithmic transformation:

$$\text{DNA} - \text{MG} = 3 \times \lg(2cDI + 1)/\lg 51 = 1.757 \times \lg(2cDI + 1)$$

2.4 Statistical analysis

For comparison, the Student *t* test was applied. Correlation between SUV and MTD and between SUV and (DNA-MG) was analysed using linear fit (Microcal Origin 5.0 program package).

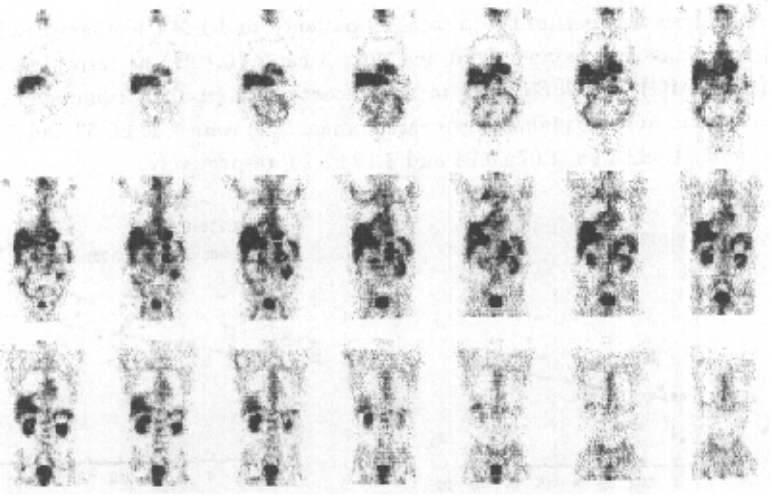

Fig.1 An intensive FDG-positive primary tumor, which was demonstrated histologically as adenocarcinoma, was showed in right lung (ϕ 1.5 cm, SUV 4.1) with increased FDG lymph node metastatic foci in the right cervical and right hilar regions. A very high FDG accumulated metastatic focus (ϕ 5.5 cm, SUV 5.5) was present in left adrenal with activity "cold" lasion in inferior area which was confirmed as central necrosis and subconjunctival hemorrhage by the surgical operation

3 RESULTS

3.1 FDG uptake

A high FDG uptake was observed in the primary tumors and metastatic lymph nodes (Fig.1 showed one case example) in all 49 patients. The values of SUV and MTD in adeno-carcinoma (n=15), squamous-cell carcinoma (n=31) and adeno-squamous-cell carcinoma (n=3) were 7.36±5.36cm and 3.54±1.74cm, 10.21±4.05 cm and 4.62±1.35cm, 12.63±6.29 cm and 4.33±1.04 cm, respectively, with no significant difference found (p >0.05) in SUVs between these histological sub-populations. The correlation between SUV and MTD was showed in Fig.2 (r=0.632, p <0.0001).

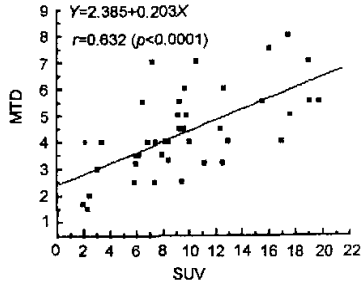

Fig.2 Correlation between SUV and MTD of primary tumor on PET scan in 49 NSCLC patients before surgery

3.2 Image cytometry

DNA analysis was performed in total 49 patients, and DNA histograms of 46 cases (93.88%) were classified as aneuploid and only 3 cases (6.12%) as tetraploid. The results of (DNA-MG) and 2cDI data in adeno-carcinoma (n=15), squamous-cell carcinoma (n=31) and adeno-squamous-cell carcinoma (n=3) were 1.43±0.53 and 7.01±5.01, 1.75±0.63 and 11.88±8.98, 1.07±0.24 and 3.19±1.20, respectively.

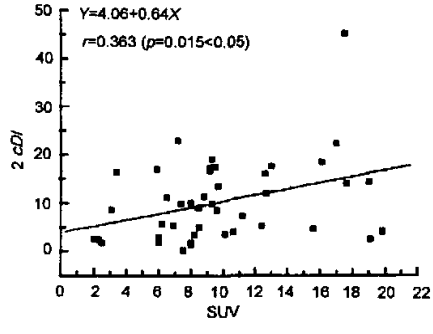

Fig.3 Correlation between SUV of primary tumor on PET and 2cDI of resected tumor in 49 NSCLC patients

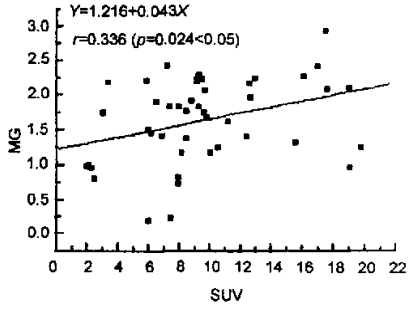

Fig.4 Correlation between SUV of primary tumor on PET and (DNA-MG) of resected tumor in 49 NSCLC patients

Statistically significant relationship was found between SUV and $2cDI$ (Fig.3), and between (SUV) and (DNA-MG) (Fig.4).

4 DISCUSSION

4.1 FDG uptake in NSCLC

Because neoplastic tissue uses glucose as an energetic substrate, ^{18}F-FDG PET can be used to visualize the presence of viable neoplastic tissue, and this modality has been shown to be a diagnostic clinical tool for preoperative staging and differential diagnosis between benign and malignant lessions, for evaluating their response to therapy and for differentiating scar tissue from residual or recurrent cancer.[5] The finding of this study of FDG PET in NSCLC that total of 49 primary lesions as well as lymph node metastasis were successfully detected prior to surgery, demonstrated the clinical usefullness of this technique. Our data of SUV v.s. MTD correlation also suggested that FDG uptake in tumor related to the number of viable tumor cells.

4.2 DNA-ploidy

Nowadays aneuploidy is regarded as a highly specific marker for the malignancy of a lesion, and it is also known to be an independent prognosticator in several malignant tumor types.[3,6,7] It has been reported by different authors that the fregnency of aneuploid tumors in lung ranges from 45%–60% starting from paraffin–embedded samples, to 80%–90% using frozen or fresh tumor material. Furthermore, (DNA-MG) as well as $2cDI$ was found to be an objective scalar index of high prognostic value in some malignant cases.[3] Filderman's group reported a DNA content study with paraffin-embedded tissues in 44 patients stage 1 NSCLC, among which 35 patients (79%) were found to be diploid tumors with 77% 5-year survival and 9 aneuploid tumors with a 44% 5-year survival.[8] In our present study, 46 of 49 NSCLC cases (93.88%) were found to have aneuploid DNA distribution pattern from fresh tumors, and the remaining 3 patients were tetraploid. The frequency of aneuploid tumors was higher than seen in other studies.

4.3 Correlation between FDG uptake and malignancy grading

In a study with rat brain tumours, Watanabe *et al.* found a positive correlation between the local cerebral glucose utilization and proliferation as measured by the bromodeoxyuridine labeling index, and they suggested that the increase in glucose utilization in these tumors is mainly needed for nucleic acid synthesis and therefore is directly related to the tumor grouth tendency.[9] Since $2cDI$ and (DNA-MG) will increase with increasing proliferation activity of the tissues, we studied the relation between FDG uptake and $2cDI$ or (DNA-MG) in this study, and revealed a correlation, but it is important to note that the slope of the regression function is flat. Large changes in FDG uptake result in

only moderate changes in the proliferation. This suggests that the glucose uptake may be not directly regulated by the needs for DNA synthesis. Barsh *et al.* found that the increase in membrane glucose transport and the size of the intracellular glucose pool are dissociated from the increase of DNA synthesis and cell growth.[10] Similar results were obtained by Weber *et al.* that the increase in glucose transport rate exceeds the requirements for growth when compared to the uptake of other growth-related substances, such as potassium and amino acids.[11] Therefore, the underlying molecular methanism for the correlation between FDG uptake and proliferation activity needs further study.

In conclusion, our study suggests, that metabolic consumption of FDG in NSCLC by ^{18}F-FDG not only is a good indicator of viable tumor cells in both primary tumor and metastases, but also provides information correlated to malignant DNA patterns which may be valuable in malignant differentiation and prognostic prediction.

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