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Alterations of tumor suppressor genes (Rb, p16, p27 and p53) and an increased FDG uptake in lung cancer

Masayuki SASAKI,* Kenji SUGIO,** Yasuo KUWABARA,* Hirofumi KOGA,* Makoto NAKAGAWA,* Tao CHEN,* Koichiro KANEKO,* Kazutaka HAYASHI,* Yoshiyuki SHIOYAMA,* Shuji SAKAI* and Hiroshi HONDA*

*Departments of *Clinical Radiology and **Surgery and Science, Graduate School of Medical Sciences, Kyushu University*

Objective: The FDG uptake in lung cancer is considered to reflect the degree of malignancy, while alterations of some tumor suppressor genes are considered to be related to the malignant biological behavior of tumors. The aim of this study is to examine the relationship between FDG-PET and alterations in the tumor suppression genes of lung cancer. ***Methods:*** We examined 28 patients with primary lung cancer who underwent FDG-PET before surgery consisting of 17 patients with adenocarcinoma, 10 with squamous cell carcinoma and 1 with large cell carcinoma. The FDG-PET findings were evaluated based on the standardized uptake value (SUV). Alterations in the tumor suppressor genes, Rb, p16, p27 and p53, were evaluated immunohistochemically. ***Results:*** The FDG uptake in lung cancer with alteration in each tumor suppressor gene tended to be higher than in those genes without alterations, although the differences were not significant. In 15 tumors with alterations in either tumor suppressor genes, the FDG uptake was 6.83 ± 3.21. On the other hand, the mean FDG uptake was 1.95 in 2 tumors without alterations in any genes. The difference in the FDG uptake between the 2 groups was statistically significant ($p < 0.001$). ***Conclusions:*** In conclusion, the presence of abnormalities in the tumor suppressor genes, which results in an accelerated cell proliferation, is thus considered to increase the FDG uptake in lung cancer.

Key words: lung cancer, FDG, PET, tumor suppressor gene

INTRODUCTION

2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) uptake in cells depends on the activity of glucose transport and/or hexokinase, and thus positron emission tomography (PET) using FDG has been used to measure glucose metabolism *in vivo*. FDG-PET is now widely used to diagnose several malignant tumors because a high FDG uptake is observed in most malignant tumors. FDG-PET is also thought to be useful for evaluating the degree of malignancy in some tumors.[1,2] In lung cancer, Higashi et al. showed a relationship between FDG uptake and the degree of cell differentiation[3,4] and the histopathological degree of aggressiveness.[5] Duhaylongsod et al. reported a correlation between FDG uptake and the tumor doubling time in lung tumors.[6] The relationship between FDG uptake and the proliferative activity of lung cancer has also been clarified by immunohistochemical analyses.[4,7] As a result, FDG uptake can possibly be used as a marker to determine the proliferative activity of lung cancer reflecting the malignant biological behavior of the tumor.

Neoplastic transformation is considered to be the result of a multistep accumulation of genetic abnormalities, including either the activation of oncogenes or inactivation of tumor suppressor genes. Recent advances in molecular biology have enabled us to identify various alterations of tumor suppressor genes in carcinogenesis. Most prominent among them are alterations in the p53

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For reprint contact: Masayuki Sasaki, M.D., Ph.D., Department of Clinical Radiology, Graduate School of Medical Sciences, Kyushu University, 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, JAPAN.

E-mail: msasaki@radiol.med.kyushu-u.ac.jp

Table 1 Patients' characteristics and the results of immunohistochemistry and FDG-PET

Pt#	Age/Sex	Histology	pStage	Immunohistochemistry				FDG (SUV)
				p53	Rb	p16	p27	
1	58 F	Adenocarcinoma	IA T1 N0 M0	Nor	Nor	Nor	Nor	2.17
2	70 F	Adenocarcinoma	IA T1 N0 M0	Alt	Nor	Nor	Nor	1.33
3	79 M	Adenocarcinoma	IA T1 N0 M0	Nor	—	—	—	2.73
4	71 M	Adenocarcinoma	IA T1 N0 M0	Alt	—	—	—	1.30
5	72 M	Adenocarcinoma	IB T2 N0 M0	Nor	Nor	Alt	Nor	4.84
6	58 F	Adenocarcinoma	IB T2 N0 M0	Nor	Alt	Nor	Nor	5.48
7	26 M	Adenocarcinoma	IB T2 N0 M0	Nor	—	—	—	3.26
8	61 M	Adenocarcinoma	IB T2 N0 M0	Nor	—	—	—	5.00
9	46 M	Adenocarcinoma	IB T2 N0 M0	Nor	—	—	—	7.77
10	52 F	Adenocarcinoma	IIB T2 N1 M0	Alt	Alt	Nor	Nor	7.16
11	59 F	Adenocarcinoma	IIB T2 N1 M0	Alt	—	—	—	5.45
12	64 M	Adenocarcinoma	IIIA T1 N2 M0	Nor	Nor	Nor	Nor	1.74
13	71 M	Adenocarcinoma	IIIA T2 N2 M0	Alt	—	—	—	5.41
14	60 M	Adenocarcinoma	IIIB T4 N2 M0	Nor	Nor	Alt	Alt	7.38
15	72 F	Adenocarcinoma	IIIB T4 N2 M0	Alt	Nor	Nor	Alt	9.59
16	64 M	Adenocarcinoma	IV T1 N1 M1	Nor	—	—	—	2.50
17	74 M	Adenocarcinoma	IV T2 N2 M1	Alt	Nor	Alt	Alt	13.13
18	78 F	Squamous cell carcinoma	IB T2 N0 M0	Alt	Nor	Nor	Alt	5.75
19	75 M	Squamous cell carcinoma	IB T2 N1 M0	Nor	Nor	Alt	Nor	4.35
20	67 M	Squamous cell carcinoma	IB T2 N0 M0	Alt	—	—	—	8.99
21	48 M	Squamous cell carcinoma	IIA T2 N2 M0	Alt	Nor	Nor	Alt	3.21
22	68 M	Squamous cell carcinoma	IIB T2 N1 M0	Nor	—	—	—	6.32
23	75 M	Squamous cell carcinoma	IIB T3 N0 M0	Nor	Nor	Nor	Alt	5.99
24	64 M	Squamous cell carcinoma	IIB T3 N0 M0	Nor	Alt	Alt	Nor	9.97
25	78 M	Squamous cell carcinoma	IIIB T4 N1 M0	Alt	Alt	Nor	Alt	4.46
26	73 M	Squamous cell carcinoma	IIIB T4 N3 M0	—	Nor	Nor	Alt	11.51
27	57 F	Squamous cell carcinoma	IIIB T4 N2 M0	Nor	Alt	Nor	Nor	8.26
28	69 F	Large cell carcinoma	IIIA T2 N2 M0	Nor	—	—	—	8.75

Alt: altered expression, Nor: normal

pathway, which inhibits cyclin E-CDK2, cyclin D-CDK4/6 and other cycilns. The Rb-p16 pathway, which inhibits the cyclin D-CDK4/6, is another major source of molecular alterations. The p27 is grouped in the Cip/Kip family which also inhibits CDK. These tumor suppressor genes normally induce a G1 arrest of the cell cycle due to inhibition of cyclin-dependent kinase activity. A loss of cell cycle regulation, due to alterations of these tumor suppressor genes, results in both an accelerated cell proliferation and tumor progression. Furthermore, alterations in these tumor suppressor genes have been reported to correlate with the biological behavior of tumors.[8–12]

Both FDG uptake and the alterations of tumor suppressor genes in lung cancer are considered to be prognostic factors. With this in mind, we designed this study to examine the relationship between the FDG uptake and alterations in the tumor suppressor genes in lung cancer.

MATERIALS AND METHODS

Patients

We studied 28 patients with primary lung cancer who underwent FDG-PET before a surgical resection. The patients consisted of 19 males and 9 females. The age of the patients ranged from 26 to 79 yrs (median: 67.5, mean ± SD: 64.6 ± 11.7 yrs). Table 1 summarizes the patient characteristics. The pathological diagnoses were as follows: 17 patients had adenocarcinoma, 10 had squamous cell carcinoma and 1 had large cell carcinoma. No patient received any previous therapy before surgery.

This study was approved by the committee for the Clinical Application of Cyclotron-Produced Radionuclides at Kyushu University Hospital, and informed consent was obtained from all patients prior to the initiation of the study.

FDG-PET

After fasting for at least 4 hours, transmission scanning with a $^{68}Ge/^{68}Ga$ ring source was performed for attenuation correction. The data acquisition for 15 minutes was started 45 minutes after the administration of 188 ± 103 (37 to 374) MBq of FDG using HEADTOME III (Shimadzu Corp., Kyoto, Japan). The images were reconstructed by the filtered back projection method using combined Butterworth and Ramp filters, and 5 contiguous transaxial images were obtained. The spatial resolution

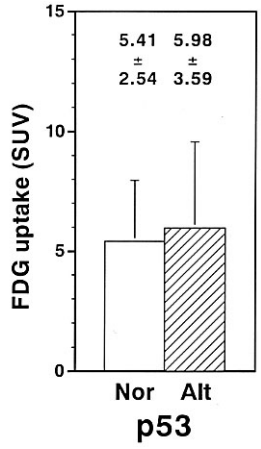

Fig. 1 The relationship between the FDG uptake and alteration in p53 expression in primary lung cancer. No significant difference was observed between the FDG uptake in tumors with or without an alteration of p53.

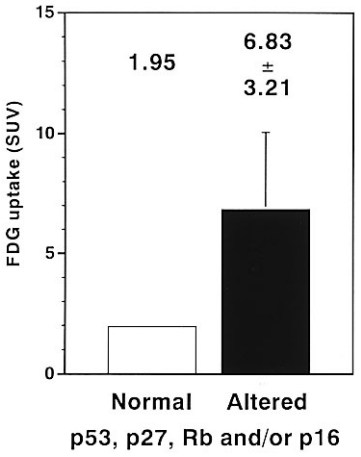

Fig. 3 The difference of FDG uptake between tumors with alteration in either Rb, p16, p27 or p53 and without alterations in any of them. The difference in the FDG uptake between the 2 groups was statistically significant ($p < 0.001$).

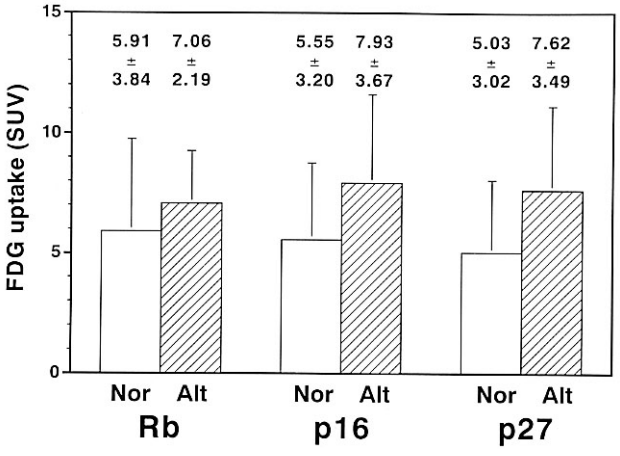

Fig. 2 The relationship between the FDG uptake and alterations in the tumor suppressor genes (Rb, p16 and p27) in primary lung cancer. No significant difference was observed between the FDG uptake in tumors with or without alterations of Rb, p16 and p27.

was 14 mm of full width at half maximum. The recovery coefficients of spherical hot phantom were 0.1, 0.8 and 0.95 at 10, 30 and 65 mm in diameter, respectively. The blood glucose level during the PET study was 95.2 ± 10.7 (71.3 to 117.7) mg/d*l*. No diabetic patients were included in this study.

For an analysis of the FDG uptake, the regions of interest (ROIs) were placed on the highest activity area in the tumor, not covering the entire tumor. The ROIs were either squares or rectangles measuring from 15 × 15 mm to 27 × 27 mm. The standardized uptake value (SUV) was then determined as the average radioactivity in the ROIs divided by the injected radioactivity normalized to the body weight.

Immunohistochemistry

Alterations of Rb, p16, p27 and p53 were examined in 17, 17, 17 and 27 patients, respectively, by using an immunohistochemical analysis as previously described.[11,13,14] Surgically resected specimens were fixed in 10% formalin and paraffin-embedded blocks were prepared. Four μm thick sections from each tissue block on slides coated with 3-aminopropyltriethoxy-sylane were de-paraffinized. For the antigenic retrieval for the antibodies, the sections were autoclaved for 5 minutes at 121°C in 0.1 M citrate buffer solution, pH 6.0. The sections were treated with 1.5% skim milk solution to reduce the non-specific absorption of antibodies. Any endogenous peroxidase activity was inhibited by treatment with 1.5% hydrogen peroxide and 100% methanol for 10 minutes. The sections were reacted with primary monoclonal antibodies diluted to 1 : 100, overnight at 4°C. Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase complex method (Histofine SAB kit, Nichirei, Tokyo, Japan). Peroxidase activity was developed with diaminobenzidine as the chromogene.

The expressions of Rb protein and p27 protein were evaluated using specific mouse monoclonal antibodies against Rb (C3-245: Pharmingen, CA, USA) and p27 (NCL-p27: Novocastra, USA). The sections were counterstained with hematoxylin. When the intensity of the stained nuclei in all areas of the lung cancer was the same as, or stronger than, the adjacent normal lung tissue, we then considered the expression level to be normal. If the staining intensity of the nucleus decreased, then it was considered to signify a reduced expression. The omission of the primary antibody resulted in a negative control. Seventeen tumors were evaluated for their expression of both Rb and p27.

The accumulation of both p53 protein and p16 protein was detected using monoclonal antibodies against p53

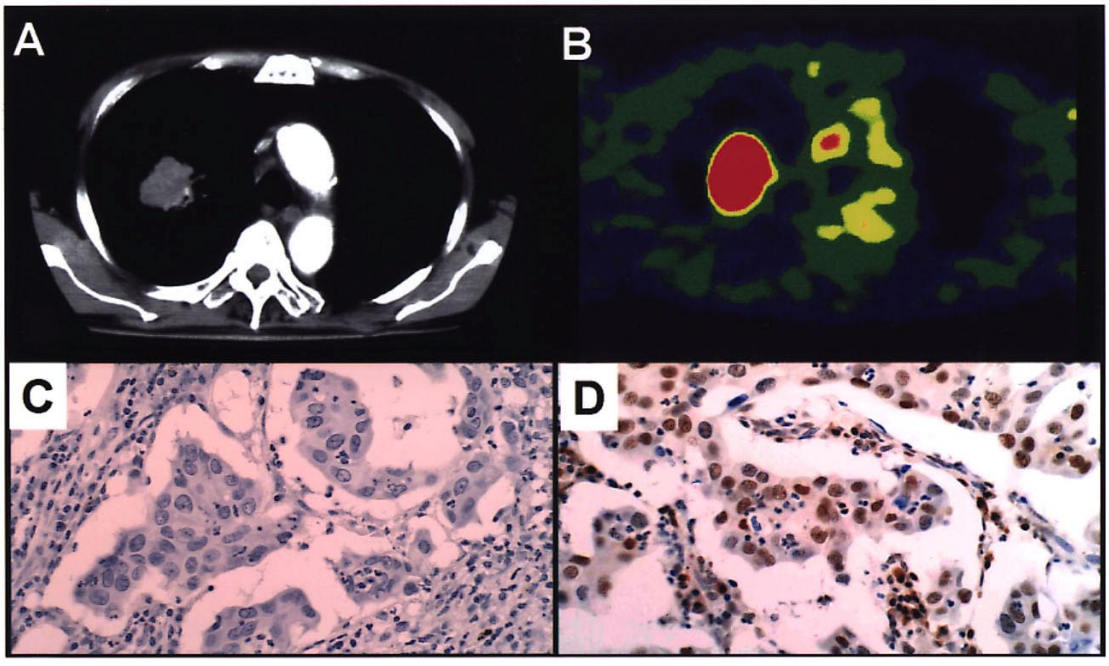

Fig. 4 Patient #17. A seventy-four-year-old male with well differentiated adenocarcinoma (Stage IV, pT2N2M1). A: CT demonstrated a tumor with 45 mm in diameter in the right upper lobe of the lung. A small lymph node measuring 4 mm in its short axis diameter is shown in the paratracheal region. B: FDG-PET demonstrated a high FDG uptake in both the primary tumor and the paratracheal region. The SUV of the primary tumor is 13.1. An FDG-PET image is presented by a fusion image which is a combination of the emission and the transmission image. An immunohistochemical analysis demonstrated a decreased Rb expression (C) and the preserved p27 expression (D).

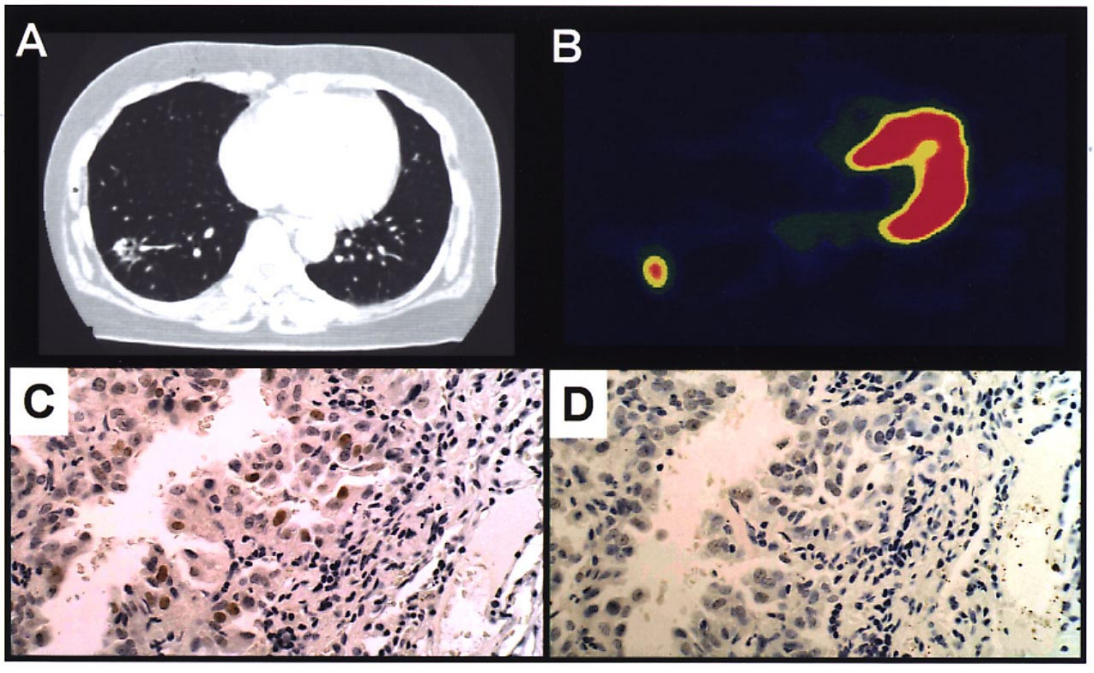

Fig. 5 Patient #1. A fifty-eight-year-old female patient with well differentiated adenocarcinoma (Stage IA, pT1N0M0). A: CT demonstrated a tumor measuring 30 mm in diameter in the right lower lobe of the lung. B: FDG-PET demonstrated an FDG uptake in the tumor (SUV = 2.17). An immunohistochemical analysis demonstrated a preserved expression of both Rb (C) and p27 (D).

(DO-7: Novocastra, Newcastle, UK) and p16 (C175-405: Pharmingen, CA, USA), respectively. The sections were counterstained with methyl green. Both the p53- and p16-labeling indices of cancer cells in each specimen were estimated by counting the number of cancer cells with a positive nucleus among at least 300 cancer cells. Carcinomas were considered to accumulate protein if 10% or more of the malignant cells contained immunohistochemistry reaction products in the nucleus.

Statistical analysis

Student's t-test and Welch's t-test were used for the statistical analyses. Probability values of $p < 0.05$ were considered to be significant.

RESULTS

Alteration of p53 expression

Immunohistochemically, the accumulation of p53 was observed in 11 of 27 tumors (Table 1). FDG uptake in tumors with and without p53 accumulation was 5.98 ± 3.59 and 5.41 ± 2.54, respectively (Fig. 1). Although the FDG uptake in tumors with altered p53 tended to be higher than in those without any alterations, no statistical significance was found in a comparison.

Alterations of Rb–CDK inhibitors

A decreased Rb expression was observed in 5 of 17 tumors, an accumulation of p16 in 5 of 17 tumors, and a decreased p27 expression in 8 of 17 tumors (Table 1). FDG uptake in tumors with a decreased Rb expression was 7.06 ± 2.19, while that with a preserved expression was 5.91 ± 3.84 (Fig. 2). FDG uptake in tumors with and without p16 accumulation was 7.93 ± 3.67 and 5.55 ± 3.20, respectively. FDG uptake in tumors with a decreased p27 expression was 7.62 ± 3.49, and in those with a preserved expression was 5.03 ± 3.02. Although FDG uptake in tumors with altered tumor suppressor genes tended to be higher than in those without any alterations, there was no statistical significance in either comparison.

Alterations of the tumor suppressor genes in either p53 or Rb–CDK inhibitors

Alternations of the tumor suppressor genes in both p53 and Rb–CDK inhibitors were examined in 17 tumors. In 15 tumors, either system was altered and the FDG uptake was 6.83 ± 3.21 (Figs. 3, 4). The mean diameter of the tumors was 53.2 ± 19.8 mm, and the recovery coefficient of HEADTOME III was 1.00 at this size. On the other hand, both systems were normal in 2 tumors, and the mean FDG uptake was 1.95 (Fig. 5). The mean diameter of the tumors was 29 mm, and the recovery coefficient was 0.77 at this size. The difference in FDG uptake between 2 groups was statistically significant ($p < 0.001$).

DISCUSSION

In this study, we examined the relationship between the FDG uptake and alteration of the tumor suppressor genes, including Rb, p16, p27 and p53, in lung cancer. The FDG uptake in lung cancer with alterations in each tumor suppressor gene tended to be higher than in their absence, however, the difference was not statistically significant. Also, the FDG uptake in lung cancer with alteration in any one of 4 tumor suppressor genes was significantly higher than when there were no alterations in any genes.

The use of a molecular biological technique enabled us to understand the multistep mechanism of carcinogenesis and to find various alterations of tumor suppressor genes. In this study, we examined the alterations in tumor suppressor genes (p53, p27, Rb and p16) based on an immunohistochemical analysis. p53 inhibits cyclin E-CDK2, cyclin D-CDK4/6 and other cycilns. On the other hand, both Rb and p16 are grouped into the "Rb pathway" which inhibits cyclin D-CDK4/6. p27 also inhibits CDK. These tumor suppressor genes normally induce a G1 arrest of the cell cycle. In lung cancer, alterations of these tumor suppressor genes are frequently observed,[8–12,15–18] and the frequency was consistent with our results.

The frequency of alterations in tumor suppressor genes in lung cancer has been reported to increase along with tumor progression, namely from preinvasive to invasive lesions,[19,20] from early to advanced stage,[21] from well to poorly differentiated lesions,[10] and from low to high stage tumors.[12,16] Furthermore, alterations of tumor suppressor genes have been considered to be an indicator of a poor prognosis.[8–12] Alterations of these tumor suppressor genes are considered to result in a loss of the cell cycle regulation, and thus could induce a biologically malignant behavior of lung cancer. Mishina et al. reported a relationship between the proliferative activity of lung cancer, as determined by the Ki-67 index, and cyclin D1 expression which correlated with an altered p53 expression.[15] Although some reports did not show any relationship between the prognosis and the alteration of single tumor suppressor genes,[17,22,23] a combined analysis further emphasized the positive relationship between the prognosis and alterations of multiple tumor suppressor genes.[8,13,18]

FDG-PET is now widely used for the diagnosis of lung cancer because a high FDG uptake is observed in malignant lesions. FDG-PET has been reported to be useful for differentiating between benign and malignant lesions,[24,25] staging[26–28] and prediction of prognosis.[5,29] Higashi et al. showed the relationship between FDG uptake and degree of cell differentiation.[3] They analyzed 29 patients with lung adenocarcinoma and also observed significant differences among the FDG uptake of bronchioloalveolar carcinoma, well differentiated carcinoma and moderately differentiated carcinoma. They also noted a relationship between the FDG uptake and the aggressiveness which is histopathologically determined when the tumor had any

one of following pathological features; pleural involvement, vascular invasion and lymphatic permeation.[5] The FDG uptake of aggressive tumors was significantly higher than that of non-aggressive ones. These reports suggest that the FDG uptake reflects the malignancy grade of lung carcinoma.

In experimental studies using either *in vitro* tumor cell lines or inoculated animal models, FDG uptake has been reported to be higher in faster-growing than in slow-growing tumors.[30,31] In clinical studies, a relationship between the FDG uptake and the proliferative activity has been reported in several tumors. Haberkorn et al. reported a correlation between FDG uptake and proliferation rate, measured by flow cytometric analysis, in head and neck squamous cell carcinoma.[32] By using immunohistochemical analysis, a correlation between the FDG uptake and the Ki-67 expression has been reported in malignant lymphoma[33] and breast cancer.[34] Jacob et al. reported a close correlation between the FDG uptake and both Ki-67 and proliferating cell nuclear antigen (PCNA) expression in head and neck cancer.[35] These data suggest that in certain tumor cell types, the glucose metabolism measured by FDG-PET varies proportionately with tumor growth. In contrast, some reports have showed no relationship between the FDG uptake and the proliferative activity based on *in vitro* experiments.[36,37] No relationship between FDG uptake and Ki-67 expression has been demonstrated either in head and neck cancer[38] or pancreas cancer.[39] Although there have been some contradictory reports regarding the relationship between FDG uptake and proliferative activity of tumor cells, recent reports have demonstrated a significant relationship between them in lung cancer using both Ki-67[4] and PCNA[7] as parameters. Clinically, a significant correlation between FDG uptake and tumor growth rate, evaluated by the tumor doubling time, was also demonstrated by Duhaylongsod et al.[6] As a result, the FDG uptake can possibly be used as a marker to determine the proliferative activity of lung cancer reflecting the malignant biological behavior of the tumor.

The FDG uptake in a small lesion is considered to be underestimated due to the partial volume effects because of the relatively low spatial resolution of our PET device.[25] The recovery coefficients of spherical hot phantoms measuring 10 mm, 35 mm and 50 mm in diameter were 0.1, 0.8 and 1.0, respectively.[40] In this study, a significantly different FDG uptake was observed between tumors without alterations in both p53 and Rb pathways and tumors with alterations in either pathway. Between these two groups, the tumor diameter was also significantly different and the recovery coefficients at each tumor size were 0.77 and 1.00, respectively. After correcting the SUVs using each recovery coefficient, the difference of the FDG uptake between the 2 groups was still statistically significant. The alteration of tumor suppressor genes is thus considered to affect the FDG uptake more than the size of the lesions.

Alterations in tumor suppressor genes have been analyzed either at the DNA or at the protein expression level. The concordance rate between gene mutations and abnormal immunohistochemistry findings have been reported to be around 60%.[9] Tumors with non-sense mutations do not show a changed protein expression. The allele loss of wild type p53 and p16 genes, resulting in a decreased protein expression, could not be detected by an immunohistochemical analysis because it does not induce a prolonged half-life of protein. On the other hand, some tumors show an abnormal protein expression without gene mutations. A DNA analysis may miss either mutations occurring outside of analyzed regions or the functional inactivation due to abnormalities in the regulator. Koga et al. reported that the heterogenous distribution of a p53 overexpression was not related to the genetic status of p53 in each tumor focus.[14] They found that some tumors with a heterogeneous p53 expression may be related to the stabilization of wild type p53 by the suppression of its regulator gene.[14] As a result, the further development of biological techniques is called for to more accurately assess the function of tumor suppressor genes.

In this study, we examined 28 patients with lung cancer. Furthermore, we analyzed only 4 tumor suppressor genes (p53, p27, Rb, p16). In addition to the above genes, the INK4 family also includes p15, p18 and p19, and Cip/Kip family includes p21 and p57. Recent advantages in molecular biology have allowed us to identify numerous oncogenes and tumor suppressor genes which may play an important role in carcinogenesis. Because carcinogenesis is considered to be the result of a multistep accumulation of genetic abnormalities, further investigations, analyzing other related genes with an increased number of subjects, may help us to better understand the mechanism of an accelerated FDG uptake in tumor cells.

CONCLUSIONS

In conclusion, the presence of abnormalities in tumor suppressor genes, which results in an accelerated cell proliferation, is considered to increase FDG uptake in lung cancer. Further investigations with an increased number of both subjects and tumor suppressor genes, may therefore help to clarify the mechanism of an accelerated FDG uptake in tumor cells.

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