RESEARCH ARTICLE

Relationships between EGFR Mutation Status of Lung Cancer and Preoperative Factors - Are they Predictive?

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Abstract

Background: The epidermal growth factor receptor (EGFR) mutation status of lung cancer is important because it means that EGFR-tyrosine kinase inhibitor treatment is indicated. The purpose of this prospective study is to determine whether EGFR mutation status could be identified with reference to preoperative factors. Materials and Methods: One hundred-forty eight patients with lung cancer (111 adenocarcinomas, 25 squamous cell carcinomas and 12 other cell types) were enrolled in this study. The EGFR mutation status of each lung cancer was analyzed postoperatively. Results: There were 58 patients with mutant EGFR lung cancers (mutant LC) and 90 patients with wild-type EGFR lung cancers (wild-type LC). There were significant differences in gender, smoking status, maximum tumor diameter in chest CT, type of tumor shadow, clinical stage between mutant LC and wild-type LC. EGFR mutations were detected only in adenocarcinomas. Maximum standardized uptake value (SUVmax:3.66±4.53) in positron emission tomography-computed tomography of mutant LC was significantly lower than that (8.26±6.11) of wild-type LC (p<0.0001). Concerning type of tumor shadow, the percentage of mutant LC was 85.7% (6/7) in lung cancers with pure ground glass opacity (GGO), 65.3%(32/49) in lung cancers with mixed GGO and 21.7%(20/92) in lung cancers with solid shadow (p<0.0001). For the results of discriminant analysis, type of tumor shadow (p=0.00036) was most significantly associated with mutant EGFR. Tumor histology (p=0.0028), smoking status (p=0.0051) and maximum diameter of tumor shadow in chest CT (p=0.047) were also significantly associated with mutant EGFR. The accuracy for evaluating EGFR mutation status by discriminant analysis was 77.0% (114/148). Conclusions: Mutant EGFR is significantly associated with lung cancer with pure or mixed GGO, adenocarcinoma, never-smoker, smaller tumor diameter in chest CT. Preoperatively, EGFR mutation status can be identified correctly in about 77 % of lung cancers.

Keywords: Lung cancer - EGFR mutation - ground glass opacity - discriminant analysis - PET-CT

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Introduction

Epidermal growth factor receptor (EGFR) mutation status of lung cancer is important because it indicates induction of EGFR-TKI (tyrosine kinase inhibitor) treatment. EGFR mutation has been reported to be strongly related with never-smoker, female, adenocarcinoma and Asians (Lynch et al., 2004; Paez et al., 2004; Pao et al., 2004). There is a striking difference in the frequency of EGFR mutations between Japanese and US patients (32% *vs* 3-9%) (Hsieh et al., 2005). If EGFR mutation status of lung cancer can be identified without molecular examination of EGFR mutation, it would be very useful for the treatment of lung cancer. Adenocarcinomas with a bronchiolo-alveolar carcinoma (BAC) component are known to be significantly correlated with EGFR mutations (Lynch et al., 2004; Pao et al., 2004; Tam et al., 2006, Sun et al., 2012). The BAC component is likely to have weak accumulation of 18F-FDG (18-fluoro-2-deoxy-glucose) in positron emission tomography-computed tomography(PET-CT)(Mori et al., 2008) and have less tissue cellularity, which is characterized by diffusion-weighted magnetic resonance imaging (DWI) (Usuda et al., 2013). PET-CT with 18F-FDG is widely accepted as an imaging modality of choice in tumor staging because of its good sensitivity (Dwamena et al., 1999; Toloza et al., 2003). The principals of DWI exploit the random motion, or so-called Brownian movement, of water molecules in biologic tissue (Le Bihan et al., 1988). Diffusion of water molecules in malignant tumors is usually restricted compared to that in normal tissue, resulting in a decreased apparent diffusion coefficient (ADC) value (Nasu et al., 2004). It is uncertain whether an examination of PET-CT or DWI is useful for the identification of EGFR mutation status.

This is a study dealing with correlations between

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preoperative factors including FDG-PET/DWI and EGFR mutation status. The purpose of this study is to investigate how much EGFR mutation status can be identified by preoperative factors including examinations.

Materials and Methods

Eligibility

The study protocol for examining DWI and PET-CT of patients with pulmonary lesions was approved by the ethical committee in Kanazawa Medical University (the approval number: No.189). A series of 148 lung cancers, in which the mutational status of the EGFR-TK domain with both reverse transcriptase-polymerase chain reaction (RT-PCR)-coupled direct sequencing and the common fragment analysis was accessible, was used for this study. Informed consent was obtained from all patients after they discussed the risks and benefits of the study with their surgeons.

Patients

Between April 2010 to December 2012, 148 patients who had operable lung cancer or who were highly suspected of having lung cancer were enrolled in this study. All of these patients were diagnosed with lung cancer before or during their operation. Eighty-two patients were male and 66 were female. Their mean age was 69 years old (range 37 to 85). UICC clinical stage of lung cancer was determined preoperatively according to the new definition of UICC 7 (International Union Against Cancer, 2009). Adenocarcinomas were classified according to the IASLC/ATS/ERS classification (Travis et al., 2011), and graded according to the histologic grading based on predominant subtype as: (1) low grade (AIS, MIA, or lepidic predominant invasive adenocarcinoma); (2) intermediate grade (papillary or acinar predominant); and (3) high grade (micropapillary or solid predominant) (Yoshizawa et al., 2011). Percentage of bronchiolo-alveolar carcinoma (BAC) in lung cancer was determined by the pathological reports of lung cancers.

PET-CT

PET-CT scanning was performed with a dedicated PET camera (SIEMENS Biograph Sensation 16, Erlangen Germany) before surgery. All patients fasted for 6 hours before scanning. The dose of ^{18}F-FDG administered was 3.7MBq/Kg of body weight. After a 60- min uptake period, an emission scan was acquired for 3 min per bed position and a whole-body scan was performed on each patient using several bed positions according to the height of each patient. After image reconstruction, a 2-dimensional (2D) round region of interest (ROI) was drawn on a slice after visual detection of the highest count on the fused CT image by the radiologist (M.T.) with 13 years of radioisotope scintigraphy and PET-CT experience who was unaware of the patients' clinical data. The maximum standardized uptake value (SUVmax) was calculated as the ^{18}F-FDG accumulation within lesions. According to CT finding, tumor shadows were classified into 3 categories based on the ratio of ground glass opacity component(GGO) within lung cancer shadow: pure GGO (not containing solid component), mixed GGO (containing solid component) and solid shadow (not containing GGO).

MR imaging

All MR images were obtained with a 1.5 T superconducting magnetic scanner (Magnetom Avanto; Siemens, Erlangen, Germany) with two anterior six-channel body phased-array coils and two posterior spinal clusters (six-channels each). DWI using a single-shot echo-planar technique was performed under SPAIR (spectral attenuated inversion recovery) with respiratory triggered scan with b value=0 and 800 s/mm^2. After image reconstruction, a 2-dimensional (2D) round or elliptical region of interest (ROI) was drawn on the lesion which was detected visually on the ADC map with reference to the T2-weghted image or CT, and ADC value of the ROI was obtained by the radiologist (H.T.) with 37 years of MRI experience who was unaware of the patients' clinical data. Areas with necrosis were excluded from the ADC measurement.

DNA extraction from paraffin-embedded tissues and EGFR mutation detection

DNA was extracted from five pieces of formalin-fixed, paraffin-embedded(FFPE) tumor tissue using the QIAamp FFPE Tissue Kit (QIAGEN KK, Tokyo, Japan). The concentration and purity of the extracted DNA was determined by spectrophotometry(NanoDrop: ND-1000). The extracted DNA was stocked at 4°C until use. EGFR mutation were analysed at SRL Co. Ltd. in Japan. The cycleave PCR technique for exons 18(G719X:G719A,G719S and G719C), 20(T790M) and 21(L858R and L861Q) of EGFR gene were used on a Thermal Cycler Dice_Real Time System TP800 (Takara Bio Inc.Shiga,Japan). The cycleave PCR technique is based on a chimeric DNA-RNA-DNA probe labeled with a fluorescent dye and quencher at each end. The RNA sequence of the probes corresponds to that of the wild type and point mutation labeled with FMA and ROX, respectively. When mutant molecules are present in the sample and PCR-amplified DNA generates a complete hybrid with the RNA portion of the mutant probe, RNase-H digests the probe at the RNA-DNA heteroduplex into two pieces, leading to a significant increase in fluorescence intensity by separation of the fluorescent dye from the quencher. The intensity of the wild-type probe served as an internal control for the assay. Deletion in exon 19 of the EGFR gene was amplified by PCR on a Thermal Cycler Dice TP600 (Takara Bio Inc.Shiga, Japan). It detected with fragment analysis using an ABI 3130xl Genetic Analyzer (Life Technologies Japan ,Tokyo, Japan). To detect the deletion and the insertion of the gene, common fragment analysis is used. Sample DNA is amplified with a FAM-labeled primer set. PCR products are electrophoresed on a sequencer. When a deletion mutation is present, PCR amplifies the shorter segment of DNA, which creates a new peak in an electropherogram.

Statistical analysis

Statistical analysis was performed using StatView for Windows (Version 5.0; SAS Institute Inc. Cary,

NC, USA). Discriminant analysis was performed using College Analysis Ver.4.0 (free software, Japan: http://www.heisei-u.ac.jp/ba/fukui/analysis.html). Differences of ratios between two groups were compared by using the χ^2 test. A two-tailed Student t test was used for comparison of differences in continuous data. A contingency table analysis was used to examine correlations between two factors with several categories. The data is expressed as the mean±standard deviation. A p value of <0.05 was considered statistically significant.

Results

There were 111 adenocarcinomas, 25 squamous cell carcinomas, 4 small cell carcinomas, 3 large cell carcinoma, 2 adenosquamous carcinomas, 1 large cell neuroendocrine carcinoma, 1 carcinoid and 1 carcinosarcoma. There were 72 clinical Stage IA (cStage IA), 36 cStage IB, 12 cStageIIA, 10 cStage IIB, 13 cStage IIIA, and 5 cStage IV. There were 5 peumonectomies, 1 bilobectomy, 110 lobectomies, 1 segmentectomy, and 31 partial resections. Three patients were treated by chemotherapy and/or radiothrerapy.

There were 58 patients with mutant EGFR lung cancers (mutant LC) and 90 patients with wild-type EGFR lung cancers (wild-type LC). Among the 58 patients with EGFR mutations, 31 had an L858R point mutation in exon21, 23 had an exon19 deletion, three had a G719X point mutation in exon18, and one had an L858R point mutation and an L861Q point mutation in exon21 multiple EGFR mutations.

There was a significant difference in gender, smoking status, maximum tumor diameter in chest CT, SUVmax, UICC clinical stage, and tumor histology between wild-type EGFR group and mutant EGFR group (Table 1). Concerning gender, the percentage of female patients (62.1%) with mutant LC was significantly higher than that of female patients (33.3%) with wild-type LC (p=0.0011). The percentage of smokers (29.3%) with mutant LC was significantly lower than that (67.8%) of smokers with wild-type LC (p<0.001). Maximum tumor diameter in chest CT (22.9±7.7) of patients with mutant LC was significantly lower than that (37.4±26.3) of patients with wild-type LC (p<0.0001). According to the type of tumor shadow, the percentage of mutant LC was 85.7% (6/7) in lung cancers with pure GGO, 65.3%(32/49) in lung cancers with mixed GGO and 21.7%(20/92) in lung cancers with solid shadows (p<0.0001). Concerning

Table 1. Patient Characteristics by EGFR Mutation Status

Characteristics		All patients, (n=148) n (%)	Wild-type EGFR, (n=90) n (%)	Mutant EGFR, (n=58) n (%)	p value
Age		68.5±8.70	69.0±9.45	67.7±7.40	0.386
Gender	Female	66 (44.6)	30 (33.3)	36 (62.1)	0.0011
	Male	82 (55.4)	60 (66.7)	22 (37.9)	
Smoking status	Smoker	78 (52.7)	61 (67.8)	17 (29.3)	<0.001
	Never-smoker	70 (47.3)	29 (32.2)	41 (70.7)	
Maximum tumor diameter in chest CT (mm)		31.8±22.3	37.4±26.3	22.9±7.7	<0.0001
Type of tumor shadow	Pure GGO	7 (4.7)	1 (1.1)	6 (10.3)	<0.0001
	Mixed GGO	49 (33.1)	17 (18.9)	32 (55.2)	
	Solid shadow	92 (62.1)	72 (80.0)	20 (34.5)	
SUV max	6.46±5.97	8.26±6.11	3.66±4.53	<0.0001	
ADC	1.26±0.34	1.24±0.33	1.29±0.35	0.4	
UICC clinical stage	IA	72 (48.6)	35 (38.9)	37 (63.8)	0.0157
	IB	36 (24.3)	21 (23.3)	15 (25.9)	
	IIA	12 (8.2)	10 (11.1)	2 (3.4)	
	IIB	10 (6.7)	10 (11.1)	0	
	IIIA	13 (8.8)	10 (11.1)	3 (5.2)	
	IIIB	0	0	0	
	IV	5 (3.4)	4 (4.4)	1 (1.7)	
Tumor histology	Adenocarcinoma (AD)	111	53	58	<0.0001
	AIS, non-mucinous	7	3	4	
	MIA, non-mucinous	5	2	3	
	Invesive AD, lepidic predominant	36	13	23	
	Invasive AD, papillary predominant	30	15	15	
	Invasive AD, acinar predominant	22	10	12	
	Invasive AD, micropapillary predominant	1	0	1	
	Invasive mucinous AD	4	4	0	
	Invasive AD solid predominant	6	6	0	
	Squamous cell carcinoma	24	24	0	
	Large cell carcinoma	3	3	0	
	Adenosquamous carcinoma	2	2	0	
	Large cell neuroendocrine carcinoma	1	1	0	
	Small cell carcinoma	5	5	0	
	Carcinoid	1	1	0	
	Carcinosarcoma	1	1	0	

*Deta for age, maximum tumor diameter in chest CT, SUV max (maximum standardized uptake value), and ADC (apparent diffusion coefficient) of lung cancer were expressed as mean±standard diviation

Table 2a. The Results of Discriminant Analysis for Correlation between EGFR Mutation Status and Clinicopathological Factors

	Discriminant function coefficients	Standardized discriminant function coefficients	p value
Tumor shadow type	1.69	0.988	0.00036
Tumor histology	1.88	0.819	0.0028
Smoking status	1.55	0.836	0.0051
Maximum diameter of tumor shadow	0.025	0.547	0.047
SUVmax	-0.044	-0.262	0.43
Clinical stage	0.077	0.113	0.69
Gender	0.062	0.031	0.91

Table 2b. Discriminant Analysis for Correlation between EGFR Mutation Status and Clinicopathological Factors

	Mutant EGFR or Wild-type EGFR	Classification Results Predicted Group Membership: Wild-type EGFR	Mutant EGFR	Total
Count	Wild-type EGFR	71	19	90
	Mutant EGFR	15	43	58
%	Wild-type EGFR	78.9	21.1	100
	Mutant EGFR	25.9	74.1	100

UICC clinical stage, clinical stage of mutant LC was significantly earlier than that of wild-type LC (p=0.0157). Concerning tumor histology, EGFR mutations were detected only in adenocarcinomas, especially in low grade or intermediate grade adenocarcinomas except a micropapillary predominant adenocarcinoma. The percentage [52.2% (58/111)] of EGFR mutations of adenocarcinomas was significantly higher than that [0% (0/37)] of non-adenocarcinomas (p=0.00014).

Concerning PET-CT, SUVmax (3.66±4.53) of mutation LC was significantly lower than that (8.26±6.11) of wild-type LC (p<0.0001) (Figure 1). Mutant percentage of EGFR were 72% (13/18) for lung cancers with SUVmax <1.0, 64% (16/25) with 1.0 ≤SUVmax <2.0, 57% (8/14) with 2.0 ≤SUVmax <3.0, 23% (2/6) with 3.0 ≤SUVmax <4.0, 32% (8/25) with 4.0 ≤SUVmax <6.0, 30% (7/23) with 6.0≤SUVmax <10, 13% (3/24) with 10 ≤SUVmax < 15, and 8% (1/13) for lung cancers with 15≤SUVmax. The more the SUVmax of lung cancer increased, the lower the mutant ratio of EGFR of lung cancer became. Concerning relationships between various exons of EGFR mutation and SUVmax of lung cancer, SUVmax was 1.35±1.20 in lung cancers with a G719X point mutation in exon18, 3.01±2.75 in lung cancers with an exon19 deletion, and 4.49±5.60 in lung cancers with an L858R point mutation in exon21(Figure 2). On the other hand, concerning DWI, ADC value (1.29±0.35) of mutant LC was same as that (1.24±0.33) of wild-type LC. ADC value of lung cancer was revealed not to be correlated to EGFR mutant status. Percentage (48.8±31.7) of BAC component in lung cancers with EGFR mutation type was significantly higher than that (14.6±26.9) of lung cancers with EGFR wild type (p<0.0001) (Figure 3).

For the results of discriminant analysis, type of tumor shadow (p=0.00036) was most significantly

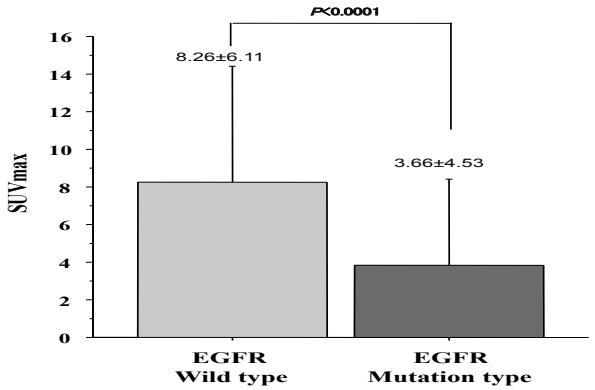

Figure 1. SUVmax of Lung Cancer Based on Status of EGFR Gene Mutation

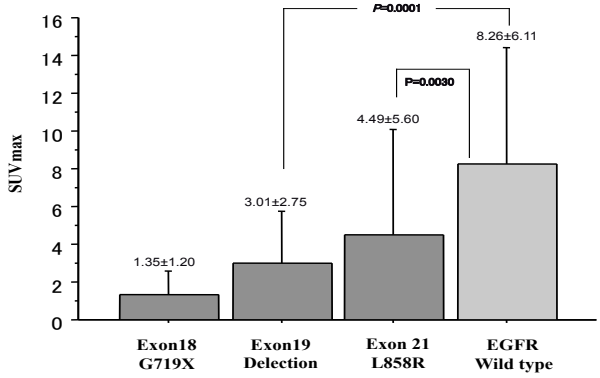

Figure 2. Relationship between EGFR Mutation and SUVmax of Lung Cancer

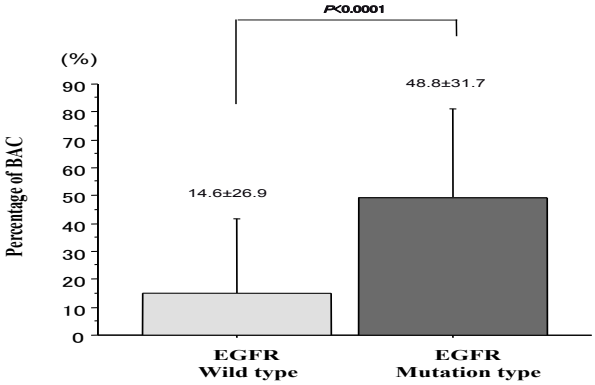

Figure 3. Percentage of Bronchiolo-Alveolar Carcinoma (BAC) Component in Lung Cancer Specimen Based on Status of EGFR Mutation

associated with mutant EGFR. Tumor histology (p=0.0028), smoking status (p=0.0051) and maximum diameter of tumor shadow in chest CT (p=0.047) were also significantly associated with mutant EGFR (Table 2a). The requested discriminant function was V=1.69x (type of tumor shadow)+1.88x (tumor histology)+1.55x (smoking status)+0.025x (maximum diameter of tumor shadow)-0.044x (SUVmax)+0.077x (clinical stage)+0.062x (gender)-9.08. Mutant EGFR was significantly associated with lung cancer with pure or mixed GGO, adenocarcinoma, never-smoker and smaller tumor diameter in chest CT. The accuracy for evaluating EGFR mutation status by discriminant analysis was 77.0% (114/148) (Table 2b).

Discussion

Recently EGFR mutation status has become one of the most important factors for selecting treatment of lung cancer. However EGFR mutation status of lung cancer cannot necessarily be examined because of inoperablility, insufficient pathological materials or cost of the molecular examination. Although EGFR mutation has been reported to be strongly related with never-smoker, female, adenocarcinoma and Asians, more detailed factors are needed to help identify EGFR mutation. Smoking status (p=0.029), N stage (p=0.021), and pathologic stage (p=0.048) were significantly associated with EGFR mutations (Liu et al. 2014). In this study, female, never-smoker, smaller maximum tumor diameter in chest CT, GGO lesion, smaller SUVmax, earlier clinical stage, and adenocarcinoma were found to be related to EGFR mutation. Lung cancer patients who fulfill these preoperative factors were likely to have EGFR mutation. The results of discriminant analysis in this study revealed that type of tumor shadow, tumor histology, smoking status and maximum tumor diameter in chest CT were significantly associated with mutant EGFR. Type of tumor shadow (differences of the GGO ratio in a CT scan) was most significantly associated with mutant EGFR. As GGO nodules noted at thin-section CT scan have been shown to have a histopathologic relationship with atypical adenomatous hyperplasia, BAC (or adenocarcinoma in situ), and adenocarcinoma with a predominant BAC component (minimally invasive adenocarcinoma) (Lee et al., 2011), EGFR mutation is positively correlated not only with the GGO ratio at a thin-section CT scan in lung adenocarcinomas (Lee et al., 2011), but also bronchioloalveolar pathologic subtype (Hsieh et al., 2005). Regarding histologic subtypes of adenocarcinoma, mixed acinar and BAC pattern showed the most frequent EGFR mutation (67.6%), followed by mixed papillary and acinar (65.2%), mixed solid and acinar (38.2%), micropapillary and acinar (30.4%), and acinar and mucinous BAC (13.3%) (Sun et al., 2012).

EGFR mutations were detected not only in 42% (5 of 12 patients) of BAC but also in 44% (4 of /9 patients) of atypical adenomatous hyperplasia (AAH) (Mclntire et al., 2010). It is generally accepted that BAC, at least in part, develops from its precursor AAH in the fashion of the adenoma-carcinoma sequence (Kitamura et al., 1999). The peripheral lung progenitor cell (Clara type 2, or other specified cell) is considered the origin of AAH and BAC (Kitamura et al., 1999). The etiology of BAC and AAH has not been clarified, as most patients with BAC and/or AAH are either non-smokers or have no history of exposure to known carcinogens (Lynch et al., 2004). Our results suggest that EGFR mutation might play an important role in the beginning of adenocarcinomatous carcinogenesis and then play a decreased role in developed lung cancer. EGFR is a key mediator of oncogenesis in non-small cell lung cancers with activation inducing tumor proliferation and growth, angiogenesis, inhibition of apoptosis, invasion, and metastasis (Jorissen et al., 2003). The majority of responses to the EGFR tyrosine kinase inhibitors gefitinib and erlotinib have been in tumors containing the BAC hislologic subtype (predominantly adenocarcinoma with BAC components) arising in patients with a history of never-smoking (Miller et al., 2004). Patients with unresectable BAC are more likely to respond to the EGFR tyrosine kinase inhibitors gefitinib and erlotinib than patients with other subtype of non-small cell lung cancer (Raz et al., 2006).

Although the discriminant analysis in this study did not reveal that PET-CT was an independent significant predictor for EGFR mutation status, SUVmax of lung cancer was revealed to be useful for prediction of EGFR mutation. High FDG activity was reported to be correlated with EGFR-wild-type genotype (Mak et al., 2011). The smaller SUVmax of PET-CT in lung cancer is, the larger the percentage of mutant EGFR is. In another multivariate analysis, a low SUV remained a significant predictor for EGFR mutations (p=0.025) (Na et al., 2010). PET-CT can be used for the identification of EGFR mutation. In the indiscriminant analysis between EGFR mutation status and preoperative factors in this study, 77% of lung cancers were revealed to be correctly classified. The result presented that EGFR mutation status can be identified by preoperative factors.

We have come to the conclusion that female, never-smoker, smaller maximum tumor diameter and GGO lesions in chest CT, smaller SUVmax, earlier stage, and adenocarcinoma were important factors which raises the possibility of EGFR mutation. Especially the type of tumor shadow is a new independent significant factor which elevates the possibility of EGFR mutation.

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