Correlation Between the Expression of EGFR and ^{18}F-FDG-PET SUVmax in Non-small Cell Lung Cancer

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ABSTRACT Objective: To investigate the relationship between the expression of EGFR (epidermal growth factor receptor) and SUVmax (standard uptake value maximum) in non-small cell lung cancer (NSCLC). Methods: 30 patients with non-small cell lung cancer and undergone PET-CT scan were registered. The two areas with different SUV max (2.5-5 vs.5 or more) of patients' tumor tissue were taken by needle puncture biopsy under the CT guidance.Expression of EGFR of the two areas were detected and analyzed by immunohistochemical staining. Results:There was no statistical difference for the expression of EGFR and SUVmax in age, gender, Pathology and differentiation (P>0.05). The expression of EGFR and SUVmax has respectively Significant difference in tumor size and clinical Stage (P<0.05). The SUVmax was correlated positively with the expression of EGFR (r = 0.836, P<0.05).Conclusions: The SUVmax is correlated positively with EGFR expression in NSCLC, which can provide the guidance for radiotherapy.

Key Words: Epidermal growth factor receptor (EGFR); Standardized uptake value (SUV); Non-small cell lung cancer (NSCLC); Immunohistochemistry

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Intrduction

Non-small-cell lung cancer (NSCLC) is one of the most common human tumors, constituting 80% of all lung tumors [1]. NSCLC is molecularly characterized by deregulations of various signaling pathways resulting from genetic alterations. Many evidences suggest that epidermal growth factor receptor (EGFR) signaling pathways are involved in the development and progression of NSCLC [2]. EGFR is frequently overexpressed in various malignant tumors, especially in NSCLC and its overexpression is associated with a poor prognosis. Positron emission tomography (PET) with 18F-fluoro-deoxyglucose (FDG) is a routine technique in the study of patients with NSCLC and it is especially useful for staging or restaging, follow-up planning radiotherapy and assessing the response to treatment [3]. This study investigated the EGFR expression in patients with NSCLC and the relation with the maximum standardized uptake value (SUVmax) in FDG-PET.

1 Material and methods

1.1 Patients

30 patients with NSCLC who were treated at The Affiliated Hospital of Qingdao University Medical College between June 2009 and December 2010 were registered. The clinical and pathological characteristics of the 30 patients were as follows: 18 (60%) were male and 12 (40%) were female; 11 (36.7%) were diagnosed as adenocarcinoma and 19 (63.3%) were diagnosed as other types of carcinoma (squamouscarcinoma, 17; Large cell carcinoma, 1; adenosquamouscarcinoma,1); 2 (6.7%) were stage I. 9(30%) were stage Ⅱ, 13(43.3%) were stage Ⅲ,and 6(20%) were stage Ⅳ. None of those patients received chemotherapy or radiotherapy before they came our hospital.

1.2 PET CT imaging protocol

All patients underwent PET CT (Siemens Biography Sensation 16) scan within 2 weeks following CT. A standard dose of 300-500 MBq of 18F-FDG was administered intravenously. PET-CT images were acquired from skull to upper thigh after an uptake period of 60 minutes. The CT component of the PET CT was performed according to a standardized protocol with the following settings: 120 kV, 100mA. Patients maintained normal shallow respiration during the CT scanning (Fig 1).The result of PET CT was regarded as negative if there was no or very low metabolic activity within lesions. A maximum standardized uptake value (SUVmax) of 2.5 was used as an arbitrary cut off.

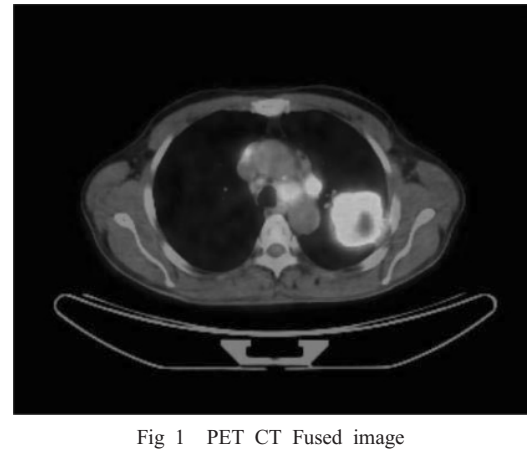

Fig 1 PET CT Fused image

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1.3 Biopsy by CT-guidance

Two areas, one with SUVmax 2.5 -5 and the other with SUVmax > 5 were selected from each patient's tumor. All lesions were imaged on a CT scanner (120 kV, 100 mA). All biopsies were performed with a 18-20 guage needle by CT-guidance. The specimens were fixed in 10% neutral buffered formalin and processed routinely (Fig 2).

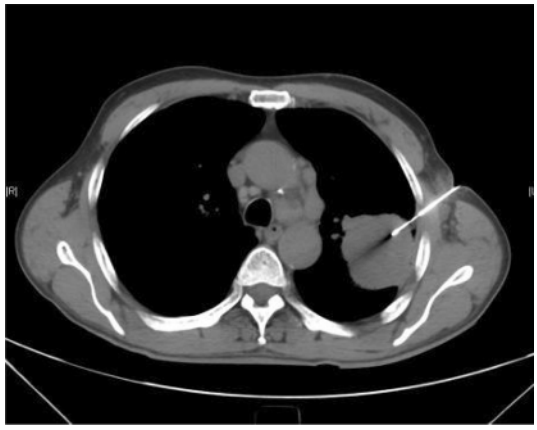

Fig 2 CT-guided percutaneous biopsy

1.4 Immunohistochemistry

In all cases, each of the formalin-fixed and paraffin-embedded tissue blocks was cut into 5 pieces spacing 2-μm and transferred to slides. One slice which was stained with HE was used for pathological diagnoses, and the others were for IHC studies.The expression levels of EGFR were observed by immunohistochemistry methods (pv-6000, two-step method). (Zhongshan Golden Bridge Biotechnology Corporation, Beijing, China) . Through enzyme digestion methods for antigen repair,PBS solution was used instead of first antibody as negative control, and Known NSCLC slices with positive expression were used as positive controls(Fig 3).

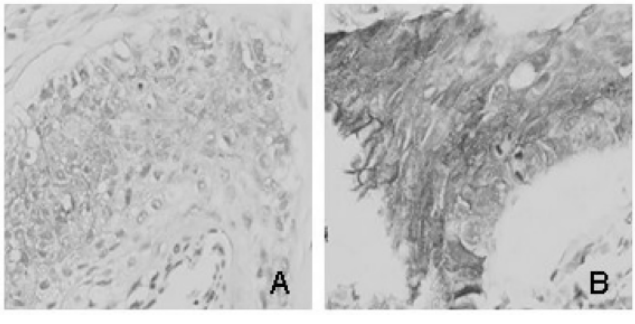

Fig 3 expression of EGFR protein in cytomembrane or cytolymph (400×):A: EGFR low expression;B: EGFR high expression

1.5 Results judgment

Two pathologists without any clinical information related to specimens performed scoring. The expression of EGFR were quantified by the method described previously published [4]. NSCLC tissues had the expression of EGFR in cytomembrane or cytolymph. Both the staining intensity and percentage of positive cells were measured. More than 5 microscopic fields (400×) in one section were counted for 100 tumor cells per field. The staining intensity was determined as the following four classes: 0 (negative) = no color; 1 (weak) = slightly yellow brown; 2 (moderate) = brown yellow; and 3 (strong) = brown. Percentage of positive cells were stratified as followed:0 for less than 10% positive cells, 1 for less than 25% positive cells, 2 for less than 50% positive cells, and 3 for more than 50% positive cells. The product of positive stained cell quantity and staining intensity was defined and calculated for each sample.

1.6 Statistical analysis

Statistical analyses were performed by using SPSS software (version 17.0). Continuous variables are shown as the Median ± standard deviation. Differences between expressions of EGFR, SUVmax and clinical pathological factors in NSCLC were examined by the Independent sample t-test. Bivariate correlation analysis was evaluated correlation expressions of EGFR with the SUVmax. $P<0.05$ was considered as statistical significant difference.

2 Results

2.1 The correlation between expression of EGFR, SUVmax and characteristics of clinical, pathological

There was EGFR immunohistochemical expression in 21 cases (70%). EGFR expression has no statistical difference in age, gender, pathology and differentiation($p>0.05$). But it has statistical significant difference in the tumor sizes and clinical Stage ($P< 0.05$). The SUVmax of the tumor has significant differences in tumor sizes and clinical Stage ($P< 0.05$). The SUVmax of the tumor has no difference in age、gender、Pathology、and differentiation ($p>0.05$)(Table 1).

2.2 The correlation between expression of EGFR and SUVmax

The SUVmax of the tumor had positive relationship with expression of EGFR ($r = 0.836$, $p = 0.000$) (Table 1). The areas that's high SVUmax had relatively high expression of EGFR, and the areas that's low SVUmax had relatively low expression of EGFR.

Table 1 Relation beteen EGFR expression and clinical pathological factors in NSCLC

	n	EGFR ($\bar{x} \pm s$)	P	n	SUV值 ($\bar{x} \pm s$)	P
Sex						
male	12	5.18+1.04		18	8.71+2.09	

female	9	6.06+0.93	0.060	12 9.90+2.73	0.188
age					
<60years	12	5.45+1.26		19 9.12+2.44	
≥ 60years	9	5.69+0.80	0.630	11 9.30+2.43	0.848
Pathology					
adenocarcinoma	7	5.00+0.91		11 8.25+2.41	
others	14	5.84+1.05	0.088	19 9.73+2.27	0.102
differentiation					
poor	7	5.69+0.82		10 10.11+2.12	
moderate-well	14	5.49+1.19	0.701	20 8.73+2.44	0.138
tumour size					
≥ 5 cm	15	5.87+0.92		20 9.93+2.41	
<5 cm	6	4.78+1.07	0.030	10 7.71+1.60	0.014
Stage					
Ⅰ-Ⅱ	8	4.64+0.89		11 7.94+2.19	
Ⅲ-Ⅳ	13	6.12+0.73	0.001	19 9.91+2.25	0.027

3 Discussion

EGFR (erbB1) is one member of the family of four erbB receptors which have a common structure comprising by an extracellular ligand-binding domain, a transmembrane domain and an intracellular domain with tyrosine kinase activity for signal transduction. Binding of the ligand such as the epidermal growth factor (EGF) or transforming growth factor α (TGFα) causes EGFR to dimerise or to heterodimerise with another member of the erbB family. This leads to receptor-linked tyrosine kinase activation and results in a signalling cascade which can produce diverse effects including cell migration, maturation, differentiation, metastasis, angiogenesis and inhibition of apoptosis[5]. The significance of tumor EGFR protein expression detected by immunohistochemistry in regard to predicting the response to targeted therapy remains to be determined. Tumor factors which can predict response to anti-EGFR therapy are still unclear. To date, the expression of EGFR in tumor such as lung cancer has not been clearly shown to predict a significant clinical response to EGFR-targeted therapy [6]. Over-expression of EGFR has been rep-orted in a wide range of human malignancies including non-smallcell lung cancer (NSCLC). Several studies have reported that expressions of EGFR were correlated with reduced survival [7], lymph node metastasis [8]and poor chemosensitivity in NSCLC [9]. While some studies had the opposite findings [10-11]. Scagliotti study showed that over-expressions of EGFR in NSCLC ranged from 40% to 80% [12]. This study has demonstrated that the expressions of EGFR had a significant correlation with tumor sizes and clinical stage respectively. The study indicates that the over-expression of EGFR protein may promote invasion and metastasis of NSCLC.

PET with ^{18}F-FDG is a routine examination in patients with tumors and is of great value in staging and, particularly, evaluating therapeutic effect. SUV is one of the parameters used to evaluate the results of PET scan in daily clinical practice. It is an indicator of the tissue concentration of the radiopharmaceutical. The SUVmax reflects cell glucose usage and acts as prognostic factors in the NSCLC [13]. FDG uptake of lung cancer cells has been reported to be more closely related to cell proliferation than to the cellular density in NSCLC[14]. Independently of their clinical impact, an attempt has been made to analyze some biological factors that can influence SUVmax. Some investigators concluded that glucose metabolism measured by 18F-FDG correlated with the degree of tumor cell differentiation of the lung cancer [15]. This study showed that when the tumor was bigger it may had a higher value of SUVmax and similar phenomena was found when the patients has advanced clinical stage. The possible reason was that tumour cell division proliferation was faster and metabolic was particularly exuberant in advanced tumors or the larger tumors, so the SUVmax was higher than others .

This study chose non-small cell lung tumors because the ^{18}F-FDG had great pract ical value [16]. Furthermore, it can also know which biochemical-molecular factors participate in the uptake of the radiopharmaceutical by the tumor. EGFR, VEGF and some enzymes of the glycolysis, hypoxia, ciclooxigenase 2 (COX 2) had been described [17-18], and because EGFR over-expressed in a very important percentage of them. This study showed a direct relationship between the expression of EGFR and SUVmax measured in the PET of patients suffering non-small cell lung cancers. Comprehensive considering the positivity relationship expression of EGFR and SUVmax, supported the important role of

cell proliferation in the uptake of the radiopharmaceutical by the tumor. This study represents to test feasibility of SUVmax-based PET-CT data on target volume delineation in planning radiotherapy of NSCLC patients, and impact of the biological target volume (BTV) delineation on IMRT treatment. The use of BTV in IMRT planning based on PET-CT is attractive because it can increase dose to targets considered to need higher doses, this may improve radiotherapy effect.

References

[1] Sharma SV, Haber DA, Settleman J. Cell line-based platforms to evaluate the therapeutic efficacy of candidate anticancer agents [J]. Nat Rev Cancer,2010,10:241-253

[2] Merrick DT, Kittelson J, Winterhalder R, et al. Analysis of c-ErbB1/epidermal growth factor receptor and c-ErbB2/HER-2 expression in bronchial dysplasia: evaluation of potential targets for chemoprevention of lung cancer [J]. Clin Cancer Res, 2006, 12: 2281 2288

[3] Melek H, Gunluoglu MZ, Demir A, et al. Role of positron emission tomography in mediastinal lymphatic staging of non-small cell lung cancer [J]. Eur J Cardiothorac Surg, 2008,33:294-299

[4] Berghmans T, Meert AP, Martin B, et al . Prognostic role of EGF-Receptor in stage III non-small cell lung cancer [J]. Eur Respir J, 2005, 25:329-335

[5] Woodburn JR. The epidermal growth factor receptor and its inhibition in cancer therapy [J]. Pharmacol Ther, 1999, 82: 241-250

[6] Niikura H, Sasano H, Matsunaga G, et al. Prognostic value of epidermal growth factor receptor expression in endometrioid endometrial carcinoma [J]. Hum Pathol, 1995, 26: 892-896

[7] Ohsaki Y, Tanno S, Fujita Y, et al. Epidermal growth factor receptor expression correlates with poor prognosis in nonsmall- cell lung cancer patients with p53 overexpression [J]. Oncol Rep, 2000,7: 603-607

[8] Fontanini G, Laurentiis MD, Vignati S, et al. Evaluation of EGF-related growth factors and receptors and of neoangiogenesis in completely resected stage I--IIIA NSCLC: amphiregulin and microvessel counts are independent prognostic indicators of survival [J]. Clin Cancer Res, 1998,4:241-249

[9] Ogawa J, Iwazaki M, Inoue H, et al. Immunohistochemical study of glutathione-related enzymes and proliferative antigens in lung cancer: relation to CDDP sensitivity[J]. Cancer, 1993,71:2204-9

[10] D'Amico TA, Massey M, Herndon JE, et al. A biologic risk model for stage I lung cancer: immunohistochemical analysis of 408 patients with the use of 10 molecular markers [J]. J Thorac Cardiovasc Surg, 1999, 117:736-743

[11] Tsai CM, Chang KT, Li L, et al. Interrelationships between cellular nucleotide excision repair, cisplatin cytotoxicity, HER-2/neu gene expression, and EGFR level in NSCLC cells [J]. Jpn J Cancer Res, 2000, 91:213-222

[12] Scagliotti GV, Selvaggi G, Novello S, et a1. The Biology of Epidermal Growth Factor Receptor in Lung Cancer [J]. Clin Cancer Res, 2004, 10 :4227-4232

[13] Berghmans T, Dusart M, Paesmans M, et al. Primary tumor standardized uptake value (SUVmax) measured on fluorodeoxyglucose positron emission tomography (FDG-PET) is of prognostic value for survival In non-small cell lung cancer (NSCLC): a systematic review and metaanalysis (MA) by the European Lung Cancer Working Party for the IASLC Lung Cancer Staging Project [J]. J Thorac Oncol, 2008,3:6-12

[14] Higashi K, Ueda Y, Yagishita M, et al. FDG PET measurement of the proliferative potential of non-small cell lung cancer[J] . J Nucl Med , 2000, 41: 85-92

[15] Higashi K, Ueda Y, Seki H, et al. Fluorine-18-FDG PET imaging is negative in bronchioloalveolar lung carcinoma [J]. J Nucl Med,1998, 39:1016-1020

[16] Maldonado A, Gonzalez-Alenda FJ, Alonso M, et al. Usefulness of positron emission tomography-computed tomography in respiratory Medicine [J]. Arch Bronconeumol, 2007,43:562-572

[17] Vesselle H, Schmidt RA, Pugsley JM, et al. Lung cancer proliferation correlates with [F18]fluorodeoxyglucose uptake by positron emission tomography [J]. Clin Cancer Res, 2000,6:3837-3844

[18] Van Baardwijk A, Dooms C, Van Suylen RJ, et al. The maximum uptake of 18F-deoxyglucose on positron emission tomography scan correlates with survival, hypoxia inducible factor 1-alpha and GLUT-1 in non-small cell lung cancer [J]. Eur J Cancer, 2007,43: 1392-1398

非小细胞肺癌 EGFR 表达与 ^{18}F-FDG-PET SUVmax 值的相关性研究

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摘要 目的:探讨非小细胞肺癌组织中 EGFR 蛋白在的表达与 PET-CT 检测的 SUVmax 的相关性。方法:临床收集 30 例非小细胞肺癌患者,每一病例首先行 PET-CT 检查,然后行 CT 导引下活检穿刺,同一病灶取 2 个穿刺部位,分别为肿瘤组织内 SUVmax 值 2.5-5 区域及 SUVmax>5 区域,采用免疫组化方法检测检测 30 例非小细胞肺癌肿瘤内部不同部位 EGFR 表达。结果:EGFR 蛋白表达、SUVmax 值在不同年龄、性别、病理类型中表达的差异无统计学意义(P 均 >0.05)。EGFR 蛋白表达、SUVmax 值在不同的肿瘤直径、临床分期组表达差异有统计学意义(P 均 <0.05)。NSCLC 肿块 SUVmax 值与组织 EGFR 表达呈显著正相关(P <0.05)。结论:NSCLC 组织 SUV 值与组织 EGFR 表达有显著相关性,可以为非小细胞肺癌的放疗提供指导。

关键词 表皮生长因子受体;最大标准摄取值;非小细胞肺癌;免疫组化

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