

Title	Detection of epidermal growth factor receptor mutations in lung adenocarcinoma cytological specimens by immunocytochemistry
Author(s)	吉田, 雅美
Citation	大阪大学, 2018, 博士論文
Version Type	
URL	https://hdl.handle.net/11094/69261
rights	
Note	やむを得ない事由があると学位審査研究科が承認したため、全文に代えてその内容の要約を公開しています。全文のご利用をご希望の場合は、 〈a href="https://www.library.osaka-u.ac.jp/thesis/#closed"〉 大阪大学の博士論文について 〈/a〉 をご参照ください。

Osaka University Knowledge Archive : OUKA

<https://ir.library.osaka-u.ac.jp/>

Osaka University

論文審査の結果の要旨及び担当者

(申請者氏名)		吉田 雅美	
論文審査担当者	(職)	氏	名
	主 査	大阪大学教授	吉井 寛一
	副 査	大阪大学教授	野々村 祝夫
	副 査	大阪大学教授	下村 伸一郎
論文審査の結果の要旨			
<p>近年上皮増殖因子受容体 (EGFR) 遺伝子変異を標的とした分子標的薬 (TKI: チロシンキナーゼ阻害剤) が開発されEGFR遺伝子変異の治療前の検出が重要となっている。現在コンパニオン診断としてバイオマーカー検査 (IVD) が用いられているが設備や技術力などによりどこでも実施可能ではなく、また肺癌の多くが手術不能な進行がんで診断されることが多く、より侵襲性の低い検体を用いた検査が求められている。今回、細胞診で陽性と診断された肺腺癌外科切除17症例について、新しい抗EGFR delE746-A750 rabbitモノクローナル抗体SP111と抗EGFR L858R抗体SP125 を用いて免疫組織化学的検査 (IHC) および免疫細胞化学的検査 (ICC) を行い、EGFR遺伝子変異との関連を調べた。スコア評価で Score 2以上をPositiveとした結果、L858R変異7例、delE746-A750変異3症例、Not mutation detected 7例において、IHCにおけるSP111, SP125抗体は感度、特異度はともに100%、一方ICCはSP125抗体は感度71.4%特異度100%であったが、SP111抗体では感度33.3%、特異度100%であった。腫瘍細胞の確保、変性を低減させるための適切な採取法と処理、保存方法、スコア評価の標準化はさらなる検討が必要であるが、非侵襲的な細胞診検体でSP111, SP125抗体を用いたICCによるEGFR変異検出は医療経済的の観点からも臨床的有用性を示唆した今回の研究は学位の授与に値すると考えられる。</p>			

論文内容の要旨

Synopsis of Thesis

氏名 Name	吉田 雅美
論文題名 Title	Detection of epidermal growth factor receptor mutations in lung adenocarcinoma cytological specimens by immunocytochemistry (肺腺癌の細胞診検体におけるEGFR変異の免疫細胞化学的検出について)
論文内容の要旨	
〔目的(Purpose)〕	
<p>Tyrosine kinase inhibitors of epidermal growth factor receptor (EGFR) improve the survival of patients with lung adenocarcinoma, and determine the EGFR mutation status before treatment is necessary. In contrast to biopsy samples, cytological specimens are obtained less invasively and are useful for EGFR mutation analyses. Recently, novel antibodies against two major EGFR mutations were developed: SP111, which is specific for the E746-A750 deletion in exon 19; and SP125, which is specific for the L858R mutation. To the best of our knowledge, no study has evaluated cytological specimens using the two novel antibodies, thus their specificity and sensitivity were examined in surgical resection, and cytological lung adenocarcinoma samples in the present study.</p>	
〔方法ならびに成績(Methods/Results)〕	
<p>Methods; This study involved 17 patients with pulmonary adenocarcinoma from whom surgical and cytological samples were obtained from January 2015 to March 2017 at Osaka University Medical Hospital. Formalin-fixed paraffin-embedded (FFPE) tissue sections (5 μm) were prepared. DNA extraction from FFPE samples was performed according to the standard procedure of the Cobas DNA Sample Preparation kit. Briefly, the samples were incubated with a protease in chaotropic lysis/binding buffer to release nucleic acids and protect genomic DNA from degradation by DNase. The amount of genomic DNA was spectrophotometrically determined and adjusted to 2 ng/μl. DNA (150 ng) was obtained for the Cobas EGFR assay. Target DNA was amplified and detected using the Cobas 4800 Analyzer according to the manufacturer's instructions. FFPE tissue sections were stained immunohistochemically using the SP111 anti-EGFR delE746-A750 rabbit monoclonal antibody and SP125 anti-EGFR L858R rabbit monoclonal antibody. The IHC staining was scored based on the staining intensity and percentage staining area in the membrane and/or cytoplasm of tumor cells as previously follows. Scores of 2+ and 3+ were considered positive.</p>	
<p>Results: Previous screening for EGFR mutation status by molecular testing identified delE746-A750 in 3 cases and the L858R mutation in 7 cases; the other cases did not have the L858R or the delE746-A750 mutation. Using a four-grade scoring system (score 0 to 3+), the immunohistochemistry (IHC) and immunocytochemistry (ICC) results were compared with those of molecular testing. Using a score of ≥ 2 as positive, IHC and ICC using SP111 demonstrated sensitivities of 100 and 33.3%, and specificities of 100 and 100%, respectively. IHC and ICC using SP125 revealed sensitivities of 100 and 71.4%, and specificities of 100 and 100%, respectively.</p>	
〔総括(Conclusion)〕	
<p>Based on the high specificity of ICC using SP111 and SP125, a positive result may eliminate the need for confirmatory molecular testing. Patients with positive cytological samples should immediately start TKI therapy without verification by molecular testing. Screening for EGFR mutations by ICC may facilitate therapeutic decision-making, particularly in medical centers unable to perform molecular testing.</p>	