学 位 論 文 の 要 旨

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学 位 論 文 名　Immunohistochemical Comparison of Biomarker Expression in Biopsy and Surgical Specimens of Non-Small Cell Lung Cancer

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論 文 内 容 の 要 旨

INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide, and the incidence has continued to increase over the last few decades. The standard chemotherapy for non-small-cell lung cancer (NSCLC) is the combination of a platinum-based and a third-generation anticancer drug.

Although researchers have identified a number of predictive and prognostic biomarkers of NSCLC, most were identified using resected tumors. Resected tumor samples cannot be obtained from patients with advanced lung cancer, however, assessment in these cases is performed using small biopsy samples. While several reports have suggested that the assessment of biomarker expression in biopsy samples is useful, it is unclear whether biomarker expression correlates with resected samples and diagnostic biopsy samples, and only a few reports have confirmed correlations in expression between surgical and biopsy samples.

In the present study, to confirm the reliability of assessment of Excision repair cross-complementing gene 1 (ERCC1), Ribonucleotide diphosphate reductase M1 (RRM1),

Thymidylate synthase (TS), and Class III beta-tubulin (BT) biomarkers by immunohistochemistry (IHC), we retrospectively reviewed lung cancer patients who were diagnosed by diagnostic biopsy and underwent surgery and evaluated these four biomarkers in resected tissue and diagnostic biopsy samples with IHC.

MATERIALS AND METHODS

The study population consisted of patients with operable and resectable NSCLC who underwent diagnostic lung biopsy and surgical resection between January 2007 and October 2010 at Shimane University Hospital, Shimane, Japan. Patients who had received chemotherapy or radiotherapy before surgery were excluded. We retrospectively identified 32 patients.

Immunohistochemical staining in both biopsy and surgical specimens was evaluated independently by four physicians who were blind to clinical data (TO, YT, AS, and TI). For ERCC1, RRM1, and TS evaluation, staining intensity was graded on a scale of 0 to 3, with higher numbers indicating higher intensity. A proportion score was assigned in four categories (0 if 0%, 0.1 if 1% to 9%, 0.5 if 10% to 49%, and 1.0 if 50% or more). This proportion score was multiplied by the staining intensity score to obtain a final semiquantitative H score. For BT evaluation, cytoplasmic expression was classified into five categories: score 0, no staining at all; score 1+, faint/barely perceptible partial cytoplasmic expression in <10% of tumor cells; score 2+, weak-tomoderate staining of the entire cytoplasm in >10% of tumor cells; score 3+, moderate staining of the entire cytoplasm in >10% of tumor cells; and score 4+, strong staining of the entire cytoplasm in >10% of tumor cells. Expression scores which were equal to or less than median values were classified as negative while those which were higher were classified as positive.

The study protocol was approved by the Ethics Committee of Shimane University and written informed consent was obtained from all subjects.

RESULTS AND DISCUSSION

ERCC1: Correlation coefficient between the biopsy and surgical specimens was r=0.512 (p=0.003), and median H score was 0.67 and 0.53, respectively. Fifteen of 32 biopsy specimens

and 16 of 32 surgical specimens were positive. Concordance rate was 78.1%.

RRM1: Correlation coefficient between biopsy and surgical specimens was r=0.411 (p=0.020), and median H score was 0.88 and 1.43, respectively. Sixteen out of 32 biopsy specimens and 14 out of 32 surgical specimens were positive. Concordance rate was 75%.

TS: Correlation coefficient between biopsy and surgical specimens was r=0.475 (p=0.006), and median H score was 1.00 and 2.00, respectively. Fifteen out of 32 biopsy specimens and 15 of 32 surgical specimens were positive. Concordance rate was 62.5%.

BT: Correlation coefficient between biopsy and surgical specimens was r=0.404 (p=0.027), and median H score was 1.00 and 2.00, respectively. Ten of 30 biopsy specimens and 14 of 30 surgical specimens were positive. Concordance rate was 53.3%.

We also compared TBLB to CT-guided lung biopsies. Larger samples can be obtained with CT-guided core biopsy over TBLB. Concordance rates in the two groups were not significantly different. Because all eligible samples were from operable cases, most tumors were so small (median: 3 cm) that any heterogeneity in the tumor was likely also small. We also compared concordance rates between T1 and T2 or more advanced stage disease. Differences were again not statistically significant. Thus, assessment of biomarker expression with biopsy samples appears to be reliable regardless of tumor size and biopsy method.

We demonstrated the feasibility of simultaneous assessment of the expression of four key predictive biomarkers in the treatment of NSCLC in samples of small size and we also confirmed a moderate correlation among them. This is the first study to identify these correlations. These findings suggest that expression of these biomarkers in biopsy samples can be reliably used in the development of individualized therapies.

CONCLUSION

We showed that a correlation exists in the expression of ERCC1, RRM1, TS, and BT in biopsy and surgical specimens in patients who had undergone surgery for NSCLC. IHC assessment can be performed in small tumor samples and provides promising data for designing individualized therapy. Introduction into clinical practice awaits retrospective confirmation of the usefulness of this analysis and the establishment of appropriate cut-off values.

論文審査及び最終試験又は学力確認の結果の要旨

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論文審査の結果の要旨

肺癌の化学療法は現在、小細胞癌とそれ以外の組織型（non-small cell lung cancer: NSCLC と略）に分けて行われているが、近年、NSCLC の中でも種々のバイオマーカーを用いて抗腫瘍薬への感受性を調べ、化学療法をより個別化する試みがされ始めている。しかし、従来は手術摘出標本を用いた検討が主体であったため、手術例にしかこのような検討がされていなかった。申請者はこれを手術できない進行癌症例に拡張するためには、生検材料で得られる情報が患者の癌組織全体を反映したものであることが必要であると考え、抗腫瘍薬の効果に影響することが知られている４つの酵素・蛋白質の発現について、同一患者の手術材料と生検材料の間で相関するか否かを、免疫染色を形態学的に評価することで検討した。32 例の肺癌患者から得られた手術材料と生検材料を用いて、Excision repair cross-complementing gene 1, Ribonucleotide diphosphate reductase M 1, Thymidylate synthase, Class III beta-tubulin（以下 tubulin と略）の 4 種類の酵素・蛋白質を免疫染色し、その染色強度を調べた。tubulin を除く３つについては、染色強度（4 段階評価）と陽性となった面積（4 段階評価）を乗じて算出した H score を用い、tubulin については、染色強度のみを用いて評価を行ったところ、生検材料と手術材料の間で有意な相関がみられた。
このことは、NSCLC における上記 4 種のマーカー発現については生検材料で癌組織全体での発現状況を推測可能であることを示唆しており、今後化学療法をさらに発展させていく上で貴重な成果である。

最終試験又は学力の確認の結果の要旨

申請者は肺癌の手術材料と生検組織を用いて免疫組織学的な検討を行い、化学療法の感受性に関係する蛋白の発現評価が生検組織を用いた検討でも手術材料を用いた検討と同様に可能であることを示した。関連領域の知識も豊富で博士の学位に値すると判定した。（主査　木下芳一）

申請者は、進行性肺癌の化学療法個別化を目標に、抗がん剤の効果に影響する 4 種の酵素・蛋白質の癌組織での発現が生検材料で推測可能であることを示した。このことは臨床的に意義のある成果である。背景の知識も充分で質疑応答も適確であったことから、学位授与に値すると判断した。
（副査　並河　徹）

申請者は肺癌のバイオマーカー候補として Excision repair cross-complementing gene 1, Ribonucleotide diphosphate reductase M 1, Thymidylate synthase 及び Class III beta-tubulin を選択し、生検検体と手術検体でこれらマーカーの発現陽性率が相関することを免疫組織学的に確認した。また、これらマーカーの組み合わせによる新たな肺がんの治療戦略の可能性を示した。関連知識も豊富で、質疑にも的確に答え、学位授与に値すると判断した。
（副査　椎名浩昭）

（備考）要旨は、それぞれ 400 字程度とする。