

行政院國家科學委員會專題研究計畫成果報告

肺癌之 telomerase 活性及其次單位基因之表現

Telomerase activity and expression of telomerase subunit genes in lung cancer

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中英文摘要

Telomerase 可維持細胞分裂後染色體之穩定，是細胞生生不死之要素，也是癌症生成之要件，以往之研究發現 telomerase 常見於癌症形成之後期，因此本研究為探討肺癌病人非癌肺組織之 telomerase 表現以及肺組織 telomerase 活性與癌轉移之相關性。本研究對象為 30 例未經治療的原發肺癌，第二及第三期，在開胸手術時分別切取肺癌組織、正常肺組織各一小塊檢查。所有組織皆經仔細之顯微鏡檢查確認，臨床資料及追蹤轉移也經仔細搜証。組織的 telomerase 活性則使用 TeloTAGGG Telomerase PCR ELISA^{plus} kit 來加以測定。並且配合臨床上及組織病理上的變化來加以分析。Telomerase 活性可以在 77% 的肺癌組織及 13% 的臨近非腫瘤組織裏偵測得到。肺癌術後再發的這組病人，他們的平均相對 telomerase 活性要沒有再發的病人組來得高(947 vs. 432, $p=0.012$)。然而，telomerase 活性和病人的年齡，性別，腫瘤範圍，淋巴結轉移，組織型態，和病理分期都沒有統計學上的相關。在追蹤期間，有 4 個病人死亡，他們的平均相對 telomerase 活性較仍存活者為高。我

們認為評估 telomerase 活性的高低，比單純偵測 telomerase 活性的有無，更有其與臨床表現的相關性。對可開刀的非小細胞肺癌而言，telomerase 活性的高低可以做為是否再發和預後的預測因子。

關鍵詞: 肺癌、癌發生、telomerase

Telomerase, a ribonucleoprotein polymerase, can compensate for the DNA end-replication problem and has been proposed to play a critical role in tumorigenesis in a lot of human malignancies. Previous studies suggested telomerase be presented at high incidence of advanced malignancies. This study was designed to disclose the incidence of telomerase activity in the noncancerous lung tissues and the correlation of metastasis and the telomerase activity of lung cancer tissues. A total of 30 patients with primary lung cancer stage II and III were enrolled in this study. The tissue specimen will be taken from the primary tumors and adjacent non-cancer lung tissues at thoracotomy. All specimens will be confirmed microscopically. The

level of telomerase activity was assessed according to the method of Kim et al with modifications using the TeloTAGGG Telomerase PCR ELISA^{plus} kit. Clinical and pathologic parameters were evaluated with respect to the level of telomerase activities. Telomerase activity was detected in 23 (77%) lung cancer tissue and 4 (13%) adjacent non-neoplastic tissue. Mean relative telomerase activity (RTA) was significant higher in the patients group with recurrence than in the patients without recurrence (947 vs. 432, $p=0.012$). However, there was no correlation between telomerase activity and age, gender, depth of tumor invasion, lymph node metastasis, histological type, and pathological staging. During the follow-up period, four patients died. The RTA of patients died was higher than that of those patients survived (mean RTA, 1020 vs. 560, $p=0.128$). Evaluation of the level of telomerase activity, rather than detection of telomerase activity, is important in evaluation the correlation between telomerase activity and clinical features. The level of telomerase activity might be a good predictor of recurrence and outcome of resectable non-small cell lung cancer.

Keywords : lung cancer, carcinogenesis, telomerase

計畫緣由及目的

Telomerase is a ribonucleoprotein

that maintains telomere base pair repeats at the ends of mammalian chromosomes during DNA replication (1,2). Telomerase activity is an important factor in the immortalization of human malignant cells (3-5). The telomerase includes three components, namely telomerase RNA component (hTR), telomerase-associated protein (TEP1), telomerase catalytic subunit (hTERT)(6, 7). The activity of telomerase is closely related to hTERT (8). Hiyama et al reported only 4.4% of non-tumor lung tissues of lung cancer revealed telomerase activity (4). But in 1999, Jong et al disclosed 79% of up-regulation in intestinal metaplasia of gastric mucosa of gastric cancer (9). Considering the theory of "field cancerization", of Slaughter (10), the adjacent normal lung tissue of lung cancer may be able to express hTERT. The main purposes of this study are to disclose the telomerase activity and expression of its subunit (hTERT, TEP1) in the lung cancer tissues and non-cancer lung tissues, which may confirm the field cancerization theory of lung cancer, and also to identify the clinical significance of telomerase activity as a marker of precancer status of lung cancer.

結果與討論

Lung carcinoma and adjacent non-neoplastic tissues were obtained from 30 patients (22 men and 8 women), ranging in age from 47 to 79 years

(mean 67.0 years) undergone operation from March to September 2000. These patients were observed clinically until September 2001. The level of telomerase activity was assessed according to the method of Kim et al with modifications using the TeloTAGGG Telomerase PCR ELISA^{plus} kit (Roche molecular Biomedical, Mannheim, Germany). Clinical and pathologic parameters were evaluated with respect to the level of telomerase activities. Telomerase activity was detected in 23 (77%) lung cancer tissue and 4 (13%) adjacent non-neoplastic tissue. Eleven patients had tumor recurrence during follow-up period. Mean relative telomerase activity (RTA) was significant higher in the patients group with recurrence than in the patients without recurrence (947 vs. 432, $p=0.012$). However, there was no correlation between telomerase activity and age, gender, depth of tumor invasion, lymph node metastasis, histological type, and pathological staging. During the follow-up period, four patients died. The RTA of patients died was higher than that of those patients survived (mean RTA, 1020 vs. 560, $p=0.128$). The level of telomerase activity tended to correlate with risk of death, although the association was not statistically significant.

Our study demonstrated that mean RTA was significant higher in the patients group with recurrence than in the patients without recurrence. The RTA

of patients died was higher than that of those patients survived. There was no correlation between telomerase activity and age, gender, depth of tumor invasion, lymph node metastasis, histological type, and pathological staging.

The major finding in our study is that evaluation of the level of telomerase activity, rather than detection of telomerase activity, is important in evaluation the correlation between telomerase activity and clinical features. Telomerase activity was one of the most important prognostic factors in the patients with non-small cell lung cancer (11). Similar correlation was reported for other types of malignancy (12-14). However, these studies used the detection of telomerase activity as the prognostic factor, which is not quantified. The telomerase activity might be positive in detection but low enough to indicate a different prognosis. Our result provided more detail in the correlation between the telomerase activity and clinical features.

計畫成果自評

Although the association was not statistically significant, the level of telomerase activity tended to correlate with risk of death. This might be due to small case number and short follow-up period. We will extend our study with larger case number and longer follow-up period to clarify this result. The telomerase subunit gene expressions are also under evaluation in our study. The

results will be integrated to the present data to understand how telomerase activity was regulated in non-small cell lung cancer.

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