行政院國家科學委員會補助專題研究計畫成果報告

探討蛋白質合成延長因子-1 在肺癌細胞株之表達和改變

Investigation the alteration of protein synthesis elongation factor-1 expressed in human lung cancer cell lines

計畫類別：X **個別型計畫**　　□**整合型計畫**

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一、中文摘要

為了瞭解蛋白質合成是否與肺癌之型態有關，我們分析了九株人類細胞株的蛋白質合成延長因子-1 的次組合體基因(α, and γ) 之表達。其中包括人類的鱗狀肺癌細胞株 CH27， Calu-1 以及腺狀肺癌細胞株 A427，H23，和 Cl-3。另外還有用皮膚基底細胞癌 BCC, 角質細胞 HaCat 以及纖維細胞 MRC5，乳癌細胞 MCF7 做為比較。此項研究計劃使用北方墨點偵測法來檢查蛋白質合成延長因子-1 的次組合體在人類肺癌細胞株之 mRNA 的表達是否有異同之處。我們發現在角質細胞 HaCat 以及乳癌細胞 MCF7 的蛋白質合成延長因子-1α基因過度表達，在人類的鱗狀肺癌細胞 Calu-1 卻只有半量表達。比較蛋白質合成延長因子-1γ的表達，HaCat 以及 MCF7 仍然過度表達，Calu-1 仍然只有半量表達。人類的鱗狀肺癌細胞株 CH27 是過度表達蛋白質合成延長因子-γ。本實驗的結果推翻以前認為轉型（transformed）細胞中蛋白質合成延長因子-1 皆會過度表達的誤解，並且證明之前報導的乳癌細胞 MCF7 中 EF-1 確實過度表達。然而人類的肺癌細胞株 A427 和 Cl-3，EF-1 之表達不受癌化影響。

關鍵詞：蛋白質合成延長因子-1、人類肺癌細胞、北方墨點偵測法。

Abstract

In order to understand the role of protein synthesis in differenciated lung cancer cell lines, we analyzed nine human cell lines that including squamous lung cancer cell lines (CH27, Calu-1) and adenocarcinoma lung cancer cell lines (A427, H23, Cl3). Other cell lines used in this study including breast adenocarcinoma cell line (MCF7), fibroblast (MRC5), keratinocyte (HaCat) and skin basal cell carcinoma (BCC). We used Northern analysis with Elongation factor-1 subunit (EF-1α, and γ) as the probe to analyze the differences in the expression of EF-1. We found that EF-1α, and γ were overexpressed in keratinocyte (HaCat) and breast cancer cell line (MCF7) but underexpressed in squamous lung cancer cell (Calu-1). The expression of EF-1γ was overexpressed in adenocarcinoma lung cancer cell (CH27). Our data did not support the reported results (Tatsuka 1992) that overexpressed EF-1 subunits were detected in the transformed cells. We also confirmed that EF-1 genes were overexpressed in breast cancer cell MCF-7 (Edmonds 1996).

Keywords: Elongation factor –1, human lung cancer, Northern analysis.

二、緣由與目的

蛋白質合成之調節是一個控制基因表達的重要步驟。蛋白質合成因子如果失控，即可以引起細胞生長極大之改變，其中包括細胞的變質和引起腫瘤的形成（Sonenberg 1993, Clemens 1999).已經證實人類的肺癌細胞株的表現與產生它們的癌組織細胞具有相當程度的一致性（Wistuba 1999）。合成延長因子-1 的次組合體 (α, β, γ, and δ) 之 mRNA 過度表達已經在數種不同的腫瘤細胞株和腫瘤組織中被報告出來（Chi 1992, Edmonds 1996, Grant 1992, Ender 1993, Mimori 1995）。可是，至今為止，人類肺癌細胞株中合成延長因子-1 是否有異常得表達還沒有研究報導。檢查合成延長因子-1 基因在人類肺癌細胞株和正常細胞株中是否有不同程度之表達可能會提供一條可靠的連線來探討肺癌細胞的發展。

此項研究計劃將使用北方墨點偵測法來檢查蛋白質合成延長因子-1 在人類肺癌細胞株之 mRNA 的表達是否有異同之處。

三、結果與討論

Overexpression of EF-1α mRNA has been correlated with increased metastatic potential in mammary adenocarcinoma (Edmonds 1996). Our data further confirmed that breast adenocarcinoma cell line (MCF7) has overexpressed not only EF-1α but also EF-1 γ mRNA. Therefore, EF-1 overexpression may be correlated with breast cancer tumorigenesis (Table 1).

Compare HaCat cells with BCC cells, a keratinocyte versus skin basal cell carcinoma;

the HaCat cells has two-fold higher EF-1α and EF-1 γ mRNA expression. Interestingly, fibroblast cell (MRC5) has similar EF-1 expression level as BCC cells. These results suggest that not all cancer cell line have higher EF-1 expression. Thus, our data did not support the reported observation that EF-1 gene determines susceptibility to transformation (Tatsuka 1992).

In the lung cancer cell lines, when we used BCC cells as the normalized standard, adenocarcinoma lung cancer cell (A427) has similar EF-1 expression as BCC cells, but squamous lung cancer cell (Calu-1) has significantly less EF-1 expression. Since CH27, another squamous lung cancer cell line has EF-1 γ overexpression, we could not conclude that EF-1 genes were repressed in squmaous lung cancer cells. We have to further characterize this observation in more detail.

We also try to analyze the expression of EF-1 genes from the cancer cell of lung tumor by collecting the pleural effusion from lung cancer patients, the EF-1 genes also observed. This preliminary data has to be carefully examined, since it has been reported that EF-1 genes mRNA levels are higher in cultured cells than tissue (Sanders 1992). The expression of EF-1 in cancer cell of pleural effusion needs to be compared with the normal cell of the same individual. We plan to carry out this study later with more collection of the sample of pleural effusion.

四、計畫成果自評

We have cloned the EF-1 genes into the bluescript SK plasmid for preparation of the probe for RNA analysis (Sheu 1992, 1997). Cell lines were obtained from different laboratories. One of the major finding for this study is to reveal the possibility of EF-1 gene activity involved in tumorigenesis.

There is a controversial issue of whether EF-1 could act as a transformation promoter. According to our data, we could not support the hypothesis of that EF-1 determines susceptibility to transformation (Tatsuka 1992). We thought that it should be more likely to see the up-regulation of EF-1 expression in cancer cell lines and human lung cancer cell lines may have up-regulation of individual EF-1 subunit, such as EF-1 γ. Surprisingly, most adenocarcinoma of lung cancer cell lines did not alter EF-1 expression dramatically. Only Calu-1 showed significantly reduced EF-1 RNA when comparing it with other cancer cell lines. The ability of EF-1 to regulate the cell proliferation and transformation apparently differs with tissue specificity.

五、參考文獻

1 Chi K, Jones DV, Frazier ML: Expression of an elongation factor 1 gamma-related sequence in adenocarcinomas of the colon. **Gastroenterology** 103: 98-102, 1992.

2 Clemens MJ., Bommer UA. Translational control: the cancer connection **Int J. Biochem Cell Biol** 31: 1-23, 1999

3 Edmonds BT, Wyckoff J, Yeung YG, Wang Y, Stanley ER, Jones J, Segall J, Condeelis, J: Elongation factor-1 alpha is an overexpressed acting binding protein in metastatic rat mammary adeno-carcinoma. **J of Cell Science** 109: 2705-14, 1996.

4 Ender B, Lynch P, Kim YH, Inamdar NV, Cleary KR, Frazier ML: Overexpression of an elongation factor-1gamma-hybridizing RNA in colorectal adenoma. **Molecular Carcinogenesis** 7:18-20, 1993.

5 Grant AG, Flomen RM, Tizard ML, Grant DA: Differential screening of a human pancreatic adenocarcinoma lambda ge11 expression library has identified increased transcription of elongation factor EF-1 alpha in tumor cells. **International Journal of Cance**r 50: 740-745, 1992.

6 Mimori K, Mori M, Inoue H, Ueo H, Mafune K, Akiyoshi T, Sugimachi K: Elongation factor 1 gamma mRNA expression in oesophageal carcinoma. **Gut** 38: 66-70, 1996.

7 Minori K, Mori M, Tanaka S, Akiyoshi T, Sugimachi K: The overexpression of elongation factor 1 gamma mRNA in

gastric carcinoma. **Cancer** 75:1446 -1449, 1995.

8 Sanders J, Maassen,J A, Möller W: Elongation factor-1 messenger-RNA levels in cultured cells are high compared to tissue and are not drastically affected further by oncogenic transformation. Nucleic Acids Res 20: 5907-5910, 1992.

9 Sonenberg N: Translation factors as effectors of cell growth and tumorigenesis. **Curr Op Cell Biol** 5: 955-960, 1993.

10 Sheu GT, Traugh JA: Recombinant subunits of mammalian elongation factor 1 (EF-1) expressed in *E. coli*; Subunit interactions, elongation activity and phosphorylation by protein kinase CK II. **J Biol Chem** 272:33290-33297, 1997

11 Sheu GT, Traugh JA: Nucleotide sequence of a rabbit cDNA encoding elongation factor 1 gamma. **Nucleic Acids Research** 20:5894, 1992

12 Tatsuka M, Mitsui H, Wada M, Negata A, Nojima H, Okayama H: Elongation factor-1 α gene determines susceptibility to transformation. **Nature** 359: 333-336, 1992.

13 Wistuba I. I., Bryant D, Behrens C., Milchgrub S., Virmani A. K., Ashfaq R., Minna, J. D., and Gazdar A. F. Comparison of features of human lung cancer cell lines and their corresponding tumors. **Clinical Cancer Research** 5: 991-1000. 1999.

Table 1

Expression level of EF-1 genes calibrated with GADPH and normalized with BCC cells.

	Calu-1	H23	MRC5	BCC	A427	CH27	Cl3	HaCat	MCF7
EF-1α	0.52	0.93	0.91	1.00	1.04	1.08	1.26	2.36	2.61
EF-1 γ	0.59	0.68	0.82	1.00	1.09	2.15	0.78	0.78	2.67