行政院國家科學委員會補助專題研究計畫成果報告

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※ 分析肺癌 Neuropilin-1 及 Semaphorin-3A 的表現與腫瘤血管新生、轉移及病人預後的相關性 ※

※ Correlation of Neuropilin-1 and Semaphorin-3A mRNA and protein expression with tumor angiogenesis, metastasis, survival and timing of relapse in lung cancer ※

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計畫類別：■個別型計畫　　□整合型計畫

計畫編號：NSC90－2314－B－002－252

執行期間：90 年 8 月 1 日至 91 年 7 月 31 日

計畫主持人：施 金 元

共同主持人：楊 泮 池

計畫參與人員：

本成果報告包括以下應繳交之附件：

- □赴國外出差或研習心得報告一份
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- □出席國際學術會議心得報告及發表之論文各一份
- □國際合作研究計畫國外研究報告書一份

執行單位：國立台灣大學醫學院內科

中　華　民　國　九十一　年　十二　月　二十六　日

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分析肺癌 Neuropilin-1 及 Semaphorin-3A 的表現與腫瘤血管新生、轉移及病人預後的相關性

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一、中文摘要

目前肺癌已是台灣以及世界各國最嚴重的癌症死亡原因。癌轉移是目前外科手術、放射治療、化學治療，各種治療方法失敗的主要原因。我們希望研究癌轉移機制，尋找與肺癌轉移有關的基因，來改善治療的方法。實驗及臨床結果皆發現，angiogenesis 對腫瘤的擴大及轉移有重大的影響。我們可以用組織免疫染色的方法，來定量微小血管的數目。經由這樣的研究，發現微小血管的數目，與肺癌病人的癌症分期與預後有相當的關聯性。最近有學者發現，neuropilin-1 (NRP-1) 和 semaphorin-3A (Sema-3A) 與腫瘤的血管新生有關；進一步，有跡象顯示 NRP-1 和 Sema-3A 可能與腫瘤的侵襲性及轉移有關。以 real-time quantitative PCR 定量 NRP-1 及 Sema-3A mRNA 量證實 NRP-1 及 Sema-3A 與癌轉移的相關性。

關鍵詞：肺腺癌、轉移機轉、neuropilin-1、semaphorin-3A

Abstract

Lung cancer is the leading cause of cancer mortality in most countries, including Taiwan. Metastasis is the most feared manifestation hat defeats the present modalities of treatments, such as surgery, radiation and chemotherapy. We are interested in studying specific mechanisms involving the metastasis in lung cancer and searching for new genes associated with the invasion of lung cancer, in order to improve the treatment modality. Angiogenesis is required for growth and progression of solid tumors. Experimental and clinical evidence suggests that the process of metastasis is angiogenesis-dependent. In lung cancer, the microvessel count may correlate with tumor stage, and might be an important prognostic indicator of metastasis and death. Originally implicated in repulsive growth cone guidance in the developing nervous system, the transmembrane receptor Neuropilin-1 (NRP-1), and its soluble ligand Semaphorin-3A, have recently been suggested to modulate vascular morphogenesis. The expression of Sema-3A was inversely correlated with the invasive ability; and the expression of NRP-1 was correlated with the invasive ability. In this study, we used real-time quantitative reverse transcription polymerase chain reaction to examine the expression of NRP-1 and

Sema-3A mRNA simultaneously in NSCLC. NRP-1 and Sema-3A expression level were prognostic indicators in NSCLC.

Keywords: Lung adenocarcinoma, metastasis, neuropilin-1、semaphorin-3A

二、背景與目的

There is a constant requirement for vascular supply in solid tumors. Tumor-associated neovascularization allows the tumor cells to express their critical growth advantage. Experimental and clinical evidence suggests that the process of metastasis is angiogenesis-dependent. Angiogenesis is required for growth and progression of solid tumors, and is important for their metastasis. Neuropilin-1 is a type 1 membrane protein with three distinct function: (1) mediate cell adhesion via a heterophilic molecular interaction; (2) binds class III semaphorins, forms complex with plexin A subfamily members and mediates the semaphorin-elicited inhibitory signals into neuron; (3) binds VEGF165 regulates vessels formation.

The semaphorin family of proteins constitute one of the major cues for axonal guidance. The prototypic member of this family is Sema-3A. Sema-3A acts as a diffusible, repulsive guidance cue *in vivo* for the peripheral projections of embryonic dorsal root ganglion neurons. Sema-3A binds with high affinity to neuropilin-1 on growth cone filopodial tips. Neuropilin-1 is required for Sema-3A action initiates the signal transduction cascade leading to growth cone collapse, axon repulsion, or growth cone turning. CRMPs are essential for Sema-3A-induced, growth cone collapse. Recently, we found that expression of CRMP-1 by transfection in a highly invasive cell line suppressed the *in vitro* invasive ability and that a low expression of CRMP-1 mRNA in lung cancer tissue was significantly associated with advanced disease, distant lymph node metastasis, early post-operative relapse and shorter survival.

Furthermore, Sema-3A inhibits the motility of porcine aortic endothelial cells in an NRP1-dependent manner by disrupting the formation of lamellipodia and inducing depolymerization of F-actin. Sema-3A inhibits the binding of VEGF165 to neuropilin-1 and vice versa. VEGF165 and Sema-3A are competitive inhibitors of each other in binding, endothelial cells motility, and dorsal root ganglion collapse assays, suggesting that the two ligands have overlapping NRP1 binding sites.

In this study, we plan to use RTQ RT-PCR to examine the expression of NRP-1 and Sema-3A mRNA simultaneously in NSCLC

Specific Aims

The significance of NRP-1 and Sema-3A expression level as a prognostic indicator in NSCLC.

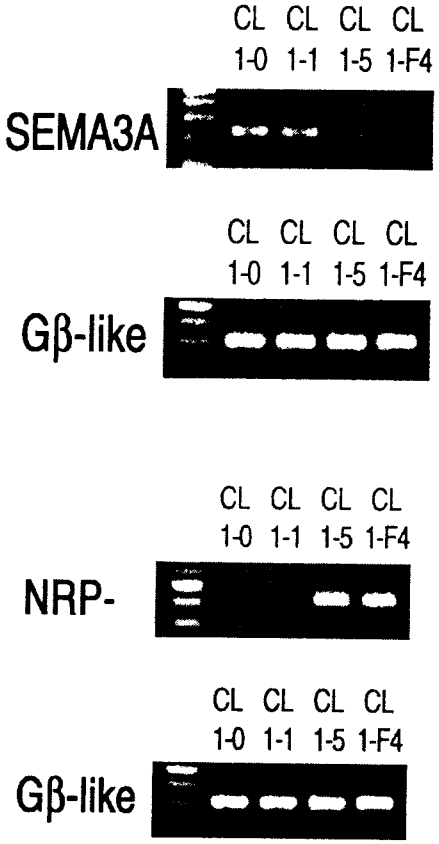
三、方法與結果

I

RT-PCR of Sema-3A and NRP-1 in cell lines that exhibit progressively increased metastatic capacities (CL1-0, CL1-1, CL1-5, and CL1--F4).

Gβ-like protein is the internal control. The figure shows that the expression of

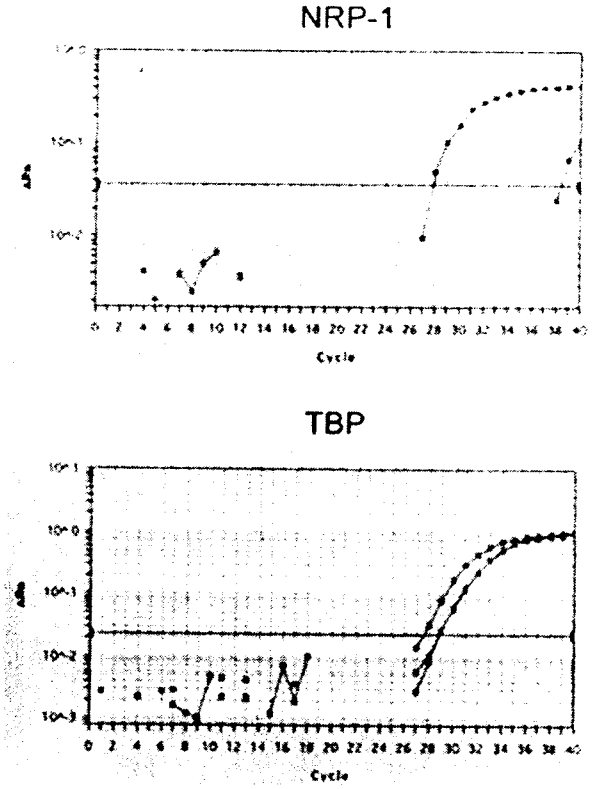
Sema-3A is inversely correlated with the invasive ability; and the expression of NRP-1 is correlated with the invasive ability.



II.

Real-time quantitative reverse transcription polymerase chain reaction of NRP-1 and TBP mRNA in cell lines that exhibit progressively increased metastatic capacities (CL1-0, CL1-1, CL1-5, and CL1--F4).

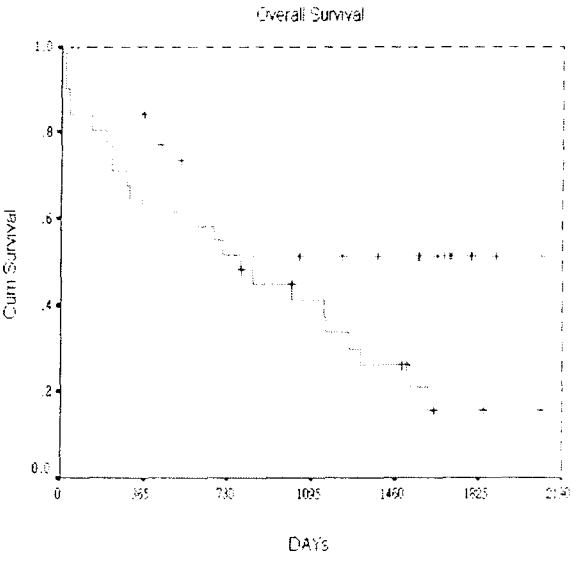
Change in normalized reporter signal (Δ Rn, y-axis) is plotted vs. cycle number (x-axis). For each reaction, the fluorescence signal of the reporter dye (FAM) is divided by the fluorescence signal of the passive reference dye (ROX), to obtain a ratio defined as the normalized reported signal (Rn). DRn represents the normalized reporter signal (Rn) minus the baseline signal. The threshold cycle (CT) value represents the fractional cycle number at which a significant increase in Rn above a chosen threshold (horizontal black line) can first be detected.

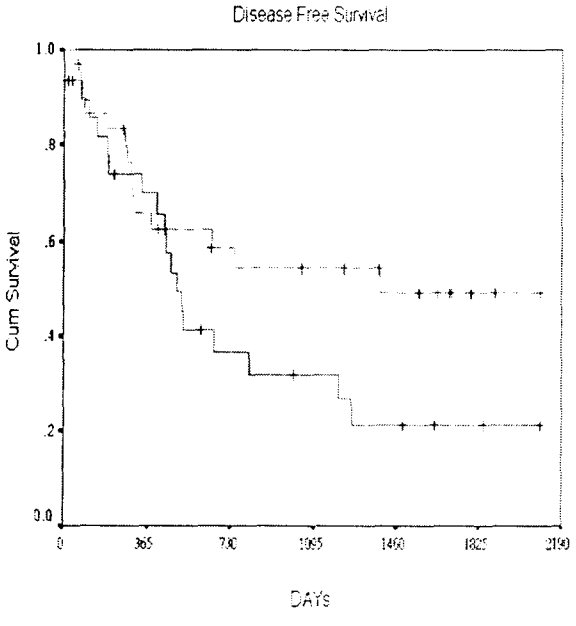


III. Kaplan-Meier survival plots for patients with nonsmall cell lung cancer, grouped according to neuropilin-1 mRNA expression.

The relative amount of tissue neuropilin-1 mRNA, standardized against the amount of TATA-box binding protein (TBP) mRNA, was expressed as $-\Delta CT = -[CT(NEUROPILIN\text{-}1) - CT(TBP)]$, where CT is the threshold cycle. Patients were included in the high-expression group if the $-\Delta CT$ was greater than the median. (A) The difference in disease-free survival between

the high- and low-expression patients is statistically insignificant (P = .10) However, there is a trend that patients with high neuropilin-1 have poor disease free survival. All patients free of recurrence at their last follow-up are indicated by tick marks on the plot. (B) The difference in overall survival between the high- and low-expression patients is statistically significant (P = .039). All patients alive at their last follow-up are indicated by tick marks on the plot.





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