行政院國家科學委員會專題研究計畫　成果報告

探討抑癌基因 Slit2 的功能與肺腺癌的關係

計畫類別：個別型計畫
計畫編號：NSC94-2320-B-040-041-
執行期間：94 年 08 月 01 日至 95 年 07 月 31 日
執行單位：中山醫學大學醫學分子毒理學研究所

計畫主持人：蔡菁華

計畫參與人員：林毓瑩，劉智雄

報告類型：精簡報告

處理方式：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中　華　民　國 95 年 10 月 31 日

行政院國家科學委員會補助專題研究計畫☑ 成 果 報 告 □期中進度報告

探討抑癌基因 slit2 的功能與肺腺癌的關係

Study the role of tumor suppressor gene Slit2 in lung adenocarcinoma

計畫類別：☑ 個別型計畫　　□ 整合型計畫

計畫編號：NSC 94-2320-B-040-041

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計畫主持人：蔡菁華

共同主持人：

計畫參與人員：林毓瑩、劉志雄

成果報告類型(依經費核定清單規定繳交)：☑精簡報告　□完整報告

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

□赴大陸地區出差或研習心得報告一份

□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

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢

□涉及專利或其他智慧財產權，□一年☑二年後可公開查詢

執行單位：中山醫學大學

中　華　民　國　95　年　10　月　25　日

附件二

可供推廣之研發成果資料表

□ 可申請專利　□ 可技術移轉　　日期：__年__月__日

國科會補助計畫	計畫名稱：探討抑癌基因 slit2 的功能與肺腺癌的關係 計畫主持人：蔡菁華 計畫編號：NSC 94-2320-B-040-041　學門領域：BE
技術/創作名稱	
發明人/創作人	
技術說明	中文： （100~500 字）
	英文：
可利用之產業及可開發之產品	
技術特點	
推廣及運用的價值	

※　1. 每項研發成果請填寫一式二份，一份隨成果報告送繳本會，一份送　貴單位研發成果推廣單位（如技術移轉中心）。

※　**2. 本項研發成果若尚未申請專利，請勿揭露可申請專利之主要內容。**

※　3. 本表若不敷使用，請自行影印使用。

行政院國家科學委員會專題研究計畫成果報告

探討抑癌基因 slit2 的功能與肺腺癌的關係

Study the role of tumor suppressor gene Slit2 in lung adenocarcinoma

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一、 中文摘要

Slit2 是一個分泌型的蛋白，它的受體是Robo蛋白，最初是因其具有阻止神經軸索移動的能力而被發現。我們實驗室利用一位女性肺腺癌及其附近之正常肺組織建立了兩個精簡雜交基因庫。由此基因庫裡發現了slit2 基因。利用RT-realtime PCR及基因微矩陣分析肺腺癌病人，發現slit2 於肺癌組織裡的表現被抑制了。此外, slit2 的表現在CL1系列細胞株中,隨著細胞之侵犯能力增加而減少。此系列細胞株之侵犯能力為CL1-0 < CL1-1 < CL1-5。Slit2 於CL1-5 之表現量約為CL1-1 表現量之 1%。欲了解Slit2 在肺腺癌扮演的角色，以RNA干擾反應將CL1-1裡slit2 的表現量降低後, 利用MTT分析細胞的生長速度降低了，並影響colony重疊的能力。當我們利用流式細胞儀分析細胞周期分布的結果發現，細胞分布於G_2M時期的比例增加。目前，我們已將 5kb的slit2 cDNA放入載體並表現在CL1-5 細胞中。我們將觀察當CL1-5 之slit2 表現量增加時，對細胞的轉移能力是否有影響。

關鍵詞：
肺腺癌、slit2、RNA 干擾

Abstract:

Slit2 is a secreted protein that binds to Robo1 and was first identified as a repellent molecule for axon guidance. Slit2 expresses in the midline of the brain. Once commissural axons pass the midline, Slit2 will project them from the midline and prevent them re-crossing the midline. Slit2 also plays important roles in many biological processes such as neuronal cell migration, leukocytes chemotaxis, muscle cell migration, etc. In the effort of identifying genes that are involved in carcinogenesis of female lung adenocarcinoma via suppressive subtractive hybridization, slit2 was identified in the subtracted cDNA libraries. Due to its remarkable biological functions, we are interested in understanding its role in carcinogenesis. RT-realtime PCR analyses showed that Slit2 is highly repressed in lung cancers in the range of 3- to 200- fold. Microarray analyses of 20 Taiwanese female lung adenocarcinomas and 5 male lung adenocarcinomas revealed that all patients but one have low Slit2 expression in lung adenocarcinoma with comparison to their normal lung counterpart (Microarray database was established by Dr. Huang CY, NHRI). Interestingly, the expression level of Slit2 is

decreased with increasing invasive ability in CL1-serjies cell lines. (a generous gift from Dr. Yang PC, National Taiwan University). The invasive ability of the CL1 series cell lines is CL1-0< CL1-1<CL1-5. The expression level of Slit2 in high invasive CL1-5 is less than 1% of that in CL1-1 by RT-realtime PCR analysis. To study the role of Slit2 in lung cancer, the expression of slit2 in CL1-1 cell line were blocked by RNA interference. To our surprise, down regulation of slit2 expression decreased growth rate of the CL1-1 cells by MTT assay and inhibited the ability to form stacking colonies. Currently, the 5kb full-length slit2 cDNA clone was expressed in CL1-5 cell line. We will examine the effect of over-expression of slit2 on cell growth, colony formation, and invasion abilities.

Key words:
Lung adenocarcinoma, slit2, RNAi

Results:

To understand the molecular biology of lung adenocarcinoma in Taiwanese female patients, two subtracted cDNA libraries were established from a female lung adenocarcinoma and its adjacent normal lung tissue. About 20 genes were selected as the result of a series of analyses. Among these genes, slit2 stood out due to its prominent biological function and highly suppressed in lung cancers. According to microarray, slit2 is repressed in 95% of female lung adenocarcinomas (figure 1).

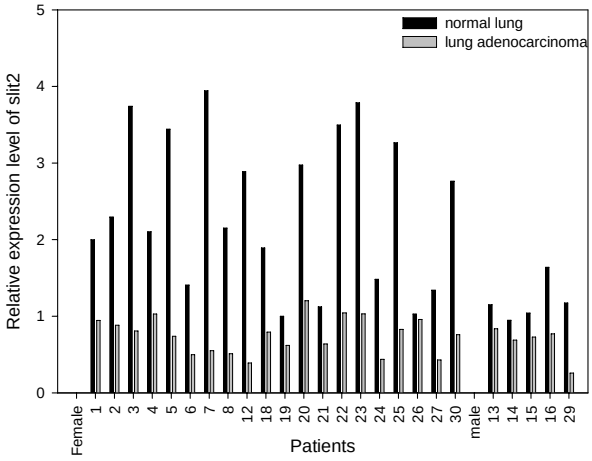


Figure 1

The Slit 2 protein is a large and diffusible extra cellular matrix glycoprotein of about 200 kD that was first identified in CNS. Slit2 is the ligand of roundabout (robo) and is expressed in the midline of the brain in *Drosophila.* Slit proteins not only mediate axon guidance in vertebrates but also direct neuron cell migrations and positively regulate axon branching. In non-neural cell, Slit2 inhibits leukocytes chemotaxis induced by chemotactic

factors, and dendritic cell migration. Thus, slit2 also function as an anti-inflammatory factor for initiating immune responses. In chicken, Slits and Robos are expressed in migrating myoblasts and neuronal projection boundaries. A recent study revealed that Slit-Robo plays a role in angiogenesis. On the other hand, slit2 has also been demonstrated to possess tumor suppressor activity.

By examining the expression of slit2 in various lung cancer cell lines, it is found that the expression of slit2 is decreased with increasing invasive ability in a CL-1 series of lung cancer cell lines (Figure 2). CL1-0, CL1-1 and CL1-5 cell lines were established by Dr. Yang P.C. (National Taiwan University) with increasing invasive ability via *in vitro* invasion screening. Since Slit2 plays an important role in regulating cell migration in many systems, and since expression of slit2 mRNA is down regulated in high invasive lung cancer cell lines, we attempt to hypothesize that slit2 may play a role in metastasis of lung cancer.

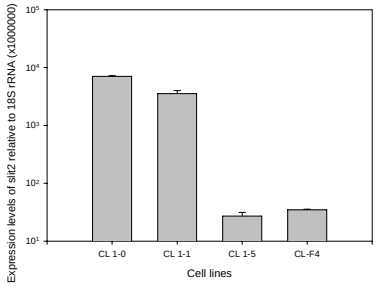


Figure 2

To explore the role of slit2 in lung tumorigenesis, we applied RNAi technique to knock down slit2 expression in CL1-1 CL1-1 cell lines that expresses high level of slit2 (Figure 3). To our surprise, knock down more than 90% of slit2 expression reduced cell growth by MTT assay (Figure 4) and showed G2/M arrest in cell cycle (Figure 5). Furthermore, the cell morphology is changed that slit2 RNAi cells forms flat colonies (except for R10) instead of stacking colonies formed in parental CL1-1 cells (Figure 6).

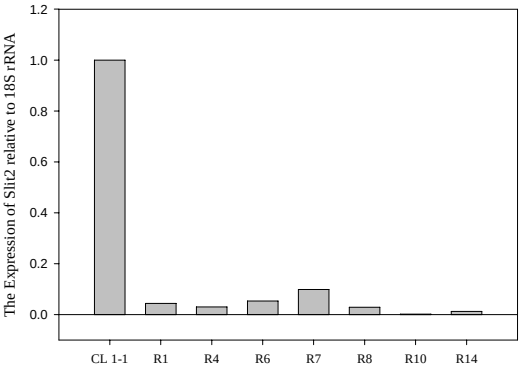


Figure 3

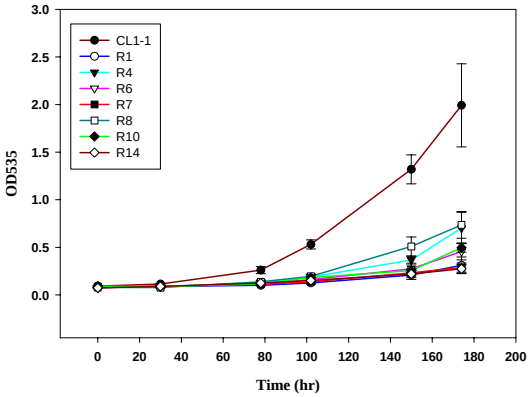
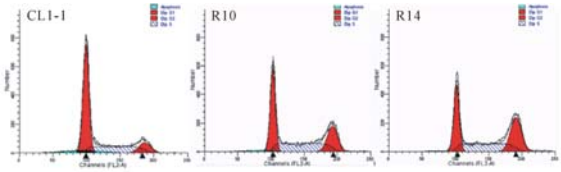


Figure 4



	CL1-1	R10	R14
G0-G1	60.5	39.5	32.7
S	28.0	36.2	33.8
G2/M	11.6	24.2	33.5
subG1	7.7	2.9	1.1

Figure 5

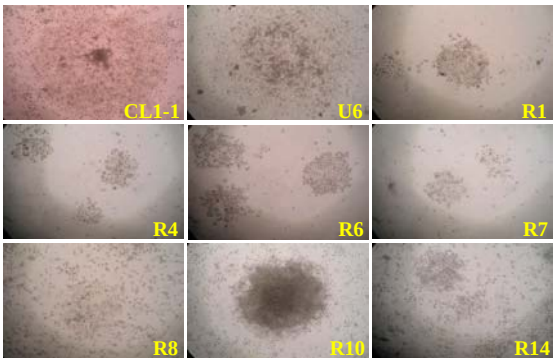


Figure 6

We are currently over-expressing slit2 in CL1-5 cells. This cell line produces low level of slit2 and possesses highest invasive ability. We will analyze the effect of over-expressed slit2 on cell growth, colony formation, and invasive abilities in CL1-5 cell line.

Discussions:

Slit/Robo signaling plays important roles in many aspects of physiological processes and pathological processes. Since there are three Slit proteins, four Robo receptors and many srGAPs, it may compose complicated signaling pathways. In this study, we will investigate the role of Slit2 signaling pathway in lung cancer cell lines. Since the CL1 series cell lines possess different invasive abilities and since the expression levels of slit2 in these cell lines are dramatically different, thus it is a nice model to study whether and how Slit2 signaling pathway may affect invasive ability and cell growth.

We used RNAi and overexpression of slit2 to study its role in lung cancer growth. Once the biological roles of slit2 in CL1-series cell lines are established, we will start to investigate the signaling pathway of slit2 involved in lung tumorigenesis.