行政院國家科學委員會專題研究計畫 成果報告

探討 Slit2 基因在肺腺癌中扮演的角色及訊息傳遞路徑(第 2 年)
研究成果報告(完整版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2320-B-040-039-MY2
執 行 期 間 ：96 年 08 月 01 日至 97 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：碩士班研究生-兼任助理人員：莊書銘
碩士班研究生-兼任助理人員：劉仲秋
碩士班研究生-兼任助理人員：張彗怡
碩士班研究生-兼任助理人員：孫仕容
博士班研究生-兼任助理人員：林毓瑩

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 97 年 09 月 05 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 Slit2 基因在肺腺癌中扮演的角色及訊息傳遞路徑

計畫類別：■ 個別型計畫　□ 整合型計畫
計畫編號：NSC 95－2320－B－040－039－MY2
執行期間：　95 年　8 月　1 日至　97 年　7 月 31 日

計畫主持人：蔡菁華
共同主持人：
計畫參與人員： 林毓瑩、莊書銘、劉仲秋、張彗怡、孫仕容

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　97 年　9　月　3　日

中文摘要

為了尋找肺腺癌中表現異常的基因，我們建立了精簡雜交基因庫，經過一連串的分析鑑定出slit2基因。利用microarray分析20位女性肺腺癌病人，發現約95%的病人其Slit2在肺癌組織的表現量低於正常組織。Slit2是一個分泌型的醣蛋白,最早被鑑定為一個具有排斥神經軸突遷徙作用的分子。之後，大部分的研究都指出Slit2在不同的組織或細胞上具有影響細胞移動的能力。然而，最近的研究發現Slit2的promoter在許多癌症組織中被高度甲基化而抑制其表現。因此，有報告指出Slit2具有抑制癌細胞生長的功能，相反的也有研究指出Slit2具有促進血管新生的功能。Slit2在大部分的肺癌細胞株中的表現量都很低，然而在CL-1系列細胞株中(kindly provided by Dr. Yang P.C., Taiwan University)，Slit2的表現情形非常有趣; 低侵犯率的CL1-0和CL1-1細胞中slit2 mRNA的表現很高，但高侵犯性的CL1-5及F4細胞中slit2的表現則很低。為了探討slit2的對肺癌細胞株的生長、侵犯性的影響，我們在CL1-5的細胞中將slit2過度表現。由於曾經有報導指出Slit2會被切割成140Kd的N-terminal片段和55-60Kd的C-terminal蛋白片段，因此本研究將全長的Slit2、N-terminal及C-terminal的蛋白片段分別表現於CL1-5細胞中。出乎意料的發現，全長的Slit2及C-terminal蛋白片段均可抑制細胞的生長，而N-terminal片段則不影響細胞的生長。過度表現全長的Slit2除了可降低細胞生長之外並可以抑制細胞在體外的移動(利用wound healing分析)及侵犯能力(利用Boydem chember分析)。意外的是Slit2的C-terminal片段也具有抑制細胞移動的能力而N-terminal片段沒什麼影響。當我們在構築Slit2基因時，發現了Slit2具有一個高度不表現exon 15的splicing variant。當exon 15存在時，全長的Slit2(Slit2-exon 15-)喪失了抑制細胞的功能。而我們所使用的Slit2 N-terminal蛋白片段具有exon 15，因此接下來必須澄清不具有exon 15的N-terminal蛋白片段是否影響細胞的生長。總結這兩年的研究結果，發現Slit2具有exon 15^+及exon 15^-兩種splicing variants，過度表現Slit2-exon 15^-的全長Slit2基因具有抑制細胞生長、移動及侵犯的能力，但exon 15^+的全長slit2基因(slit2-exon 15^+)只具有抑制細胞移動的能力而不能抑制細胞的生長。目前的結果顯示，C-terminal片段在細胞的生長與移動上扮演著重要的加色，然而exon 15的存在與否可以決定Slit2抑制生長的能力。

英文摘要

In the effort of identifying genes that participating lung tumorigenesis, we established two subtractive cDNA libraries through suppressive subtractive hybridization. After series analysis, 20 genes were identified with differential expression pattern between lung cancer tissues and its normal counter parts. Among them slit2 was stood out due to its prominent biological functions. Slit2 is a secreted glycoprotein which was first identified as a repellent molecule of axon guidance. Most biological function of Slit2 was referred to its ability of affecting cell migration in various cell types. Recently, more evidences exhibited extra function of Slit2 in carcinogenesis and angiogenesis. Slit2 is highly expressed in normal lung, but greatly repressed in lung cancer. The biological function of Slit2 in normal lung is largely unknown. Most of lung cancer cell lines express low level of slit2, but in CL1 series cell lines (via *in vitro* invasion screening, kindly provided by Dr. Yang P-C, Taiwan University), slit2 showed interesting expression patterns. The expression of slit2 is high in lower invasive cell lines CL1-0 and CL1-1, but its expression is greatly repressed in CL1-5 and F4, which possess high invasive abilities. To investigate whether Slit2 would affect lung cancer growth and invasion, Slit2 was overexpressed in CL1-5 cells. Slit2 has been reported to be cleaved into 140Kd N-terminal and 55-60Kd C-terminal fragments. Therefore, each of these constructs was over-expressed in CL1-5 for analyses. Our results showed that full-length Slit2 inhibited cell growth and this cell growth inhibition was reserved by Slit2 C-terminal fragment but not Slit2 N-terminal fragment. In addition, full-length Slit2 also inhibited cell motility (via wound healing assay) and invasive ability (via modified Boydem chamber analysis). Unexpectedly, Slit2 C-terminal fragment also had the ability to inhibit cell motility but not Slit2 N-terminal. This result conflicted with other reports that showed Slit2 N-terminal fragment possessed ability to inhibit cell migration. During Slit2 cloning process we identified exon 15^{+} (Slit2-exon 15^{+}) and exon 15^{-} (Slit2-exon 15^{-}) splicing variants. Surprisingly, when exon 15 is present in full-length Slit2, Slit2 lost its growth inhibition ability. The N-terminal fragment of Slit2 in this study also contains exon 15. Thus, we need to clarify whether Slit2 N-terminal without exon 15 would affect cell growth. In summary, we identified exon 15^{+} and exon 15^{-} splicing variants of Slit2. Overexpression of Slit2-exon 15- in CL1-5 inhibited cell growth, motility and invasive abilities, while Slit2-exon 15^{+} only reduced cell motility but not cell growth. It seems that Slit2 C-terminal fragment plays important roles in both cell growth inhibition and motility inhibition, however existence of Exon 15 or not may modulate the ability of cell growth inhibition ability of Slit2.

目錄

報告內容

一、前言:

Lung cancer is the leading cause of cancer death worldwide. Although the cigarette consumption is decreased, the incidence of lung cancer still uprises annually. Among four types of lung cancer (small cell lung cancer, large cell carcinoma, squamous cell carcinoma, and adenocarcinoma), cigarette smoke is tightly associated with small cell carcinoma and squamous cell carcinoma. Recently, adenocarcinoma becomes much more prevalence especially for the non-smokers. In Taiwan, above 90% of female lung cancer was non-smoker. The etiology of lung cancer in non-smokers was largely unknown. One way to study lung carcinogenesis is to identify genes that participates this process. Our laboratory is interested in identifying genes whose expression are changed between normal lung and female lung adenocarcinoma. To achieve the goal, we performed suppressive subtractive hybridization. After series of analyses, about 20 genes were identified with differentially expression pattern between normal lung and lung adenocarcinoma. We focus on the secreted proteins and membrane proteins for further studies.

二、研究目的:

Slit2 is a secreted protein. In neuron, Slit2 acts as an axon repellent molecule that would guide axon migration. It is highly expressed in normal lung, but repressed in lung cancer (figure 1). In this study, we performed RNAi and over-expression techniques to study the role of Slit2 protein in lung cancer cell lines. During cloning process, we found an abundant alternative splicing variant, exon 15 of slit2 mRNA, presents in most of the lung cancer cell lines. In this study, we also attempt to understand if the exon 15 plays an important role in slit2 function. Slit2 can be cleaved into two fragments, the N-terminal fragment possesses known guiding signal, however the function of C-terminal fragment remain uncover. Thus, this study would hopefully reveal possible biological functions of N- and C- terminal of Slit2 in lung.

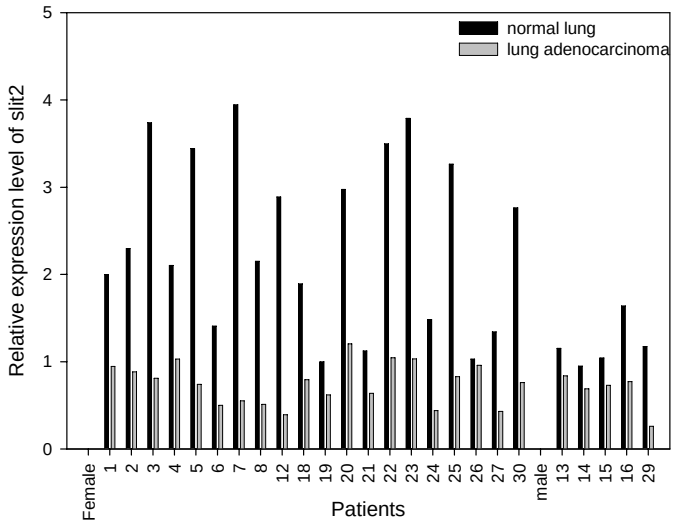


Figure 1: Relative expression level of slit2 in Taiwanese lung adnocarcinoma analyzed by microarray. Gray bars represent slit2 expression in lung adenocarcinoma, and the black bars represent normal counterpart of the same patient.

三、文獻探討:

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*. The robo gene was identified as a control element that *robo* mutant embryos produced too many axons cross and re-cross the CNS midline. Four vertebrate Robos, Robo1, Robo2, Robo3 (Rig) and Robo4, and three Slits, Slit1, Slit2 and Slit3 were identified in mammal up to now. Both Robo 1 and Robo3 are expressed on commissural neuron before crossing the midline. However, Robo3 is not expressed on the axon after crossing the midline. The scenario of commissural axons migration might be as follows: Robo3 represses the effect of the repellent role of Slit2 thus allowing commissural axons approach and across the midline. Once the axons reach the midline, Robo3 is degraded and the repellent role of Slit2 starts to work that would lead the axon away from the midline and avoid the axons re-crossing the midline.

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 functioning as an anti-inflammatory factor for initiating immune responses. In chicken, Slits and Robos are expressed in migrating myoblasts and neuronal projection boundaries. In *drosophila*, Slit also directs the movement of epithelial sheaths and controls muscle precursor cell migration during

myogenesis. Interestingly, during myogenesis slit functions switches from repulsion to attraction. Moreover, a recent study revealed that Slit-Robo plays a role in angiogenesis. In this case, Slit2 is expressed in tumor cells and it works as an attractant for endothelial cell that expresses Robo1. On the other hand, slit2 were reported to be down regulated in several cancers including lung cancers, breast cancers, gliomas, wilm's tumor, and renal cell carcinoma via hypermethylation of slit2 promoter region. Slit2 has also been demonstrated to possess tumor suppressor activity. While Slit2-Robo1 signaling promotes angiogenesis, another Robo family, Robo4 expressed primarily in endothelial cell, binds to Slit2 and inhibits endothelial migration.

Slit2 possesses multiple putative protein interaction motifs, from N-terminal including four leucin-rich repeats (LLRs), nine EGF-like repeats, a laminin G domain, and a C-terminal cycteine-rich knot. Slit2 is proteolytically processed into 140 kD N-terminal and 55-60 kD C-terminal fragments at the junction between the fifth and sixth EGF-like repeats. The N-terminal fragment and full length Slit2 binds to Robo receptor with similar affinity while the C-terminal fragment does not bind to Robo. The N-terminal fragment of Slit2 has an ability to induce sensory axon elongation and branching.

Although most of the reports pointed out that Slit-Robo signaling is related to cell motility, Slit-Robo signaling may function in promoting terminal asymmetric cell division in *Drosophila*. In summary, 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. By *in vitro* invasive screening, sublines of CL1 was isolated with various invasive ability as CL1-0 < CL1-1 < CL1-5, while F4 was screened by *in vivo* invasive screening of CL1-5 cells (kindly provided by Dr. Yang P.C., Taiwan University). The expression level of slit2 is inverse to the invasive ability of CL1 series cell line. Thus CL-1 series cell line is a nice model to study whether and how Slit2 signaling pathway may affect invasive ability and cell growth.

四、結果:

In this project, we constructed six slit2 constructs including full-length slit2 with/without Exon 15 (Exon $15^{+}/\triangle^{+}$ and Exon 15-/$\triangle^{+}$), Exon 15 deleted slit2 with/without cleavage site (Exon 15-/$\triangle^{+}$ and Exon 15-/$\triangle^{-}$), and constructs with N-terminal fragment or C-terminal fragment. All these constructs were stably expressed and secreted successfully in CL1-5 cells detected by both anti-myc and anti-Slit2 antibody (Figure 1).

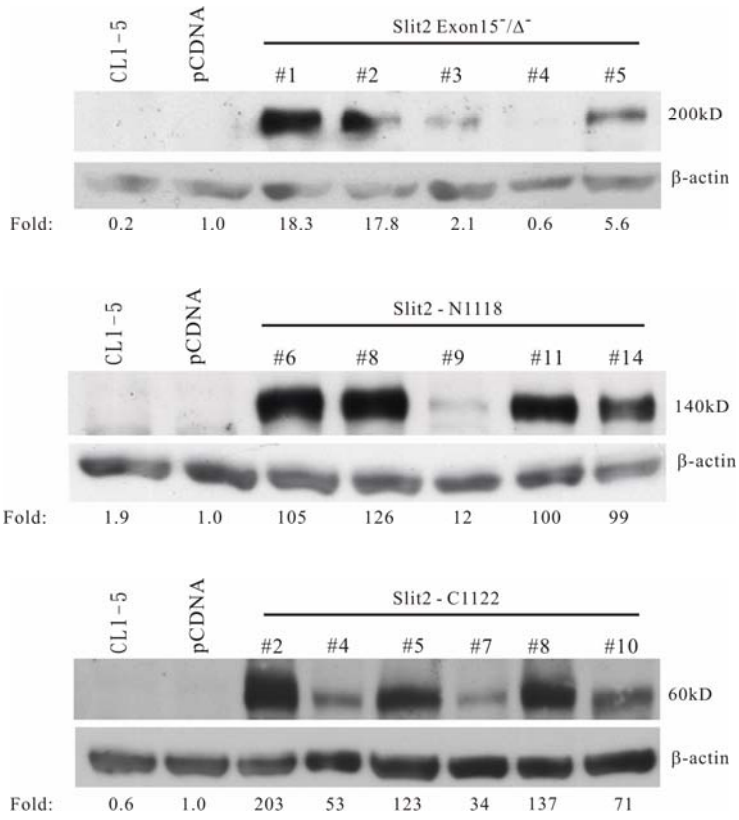
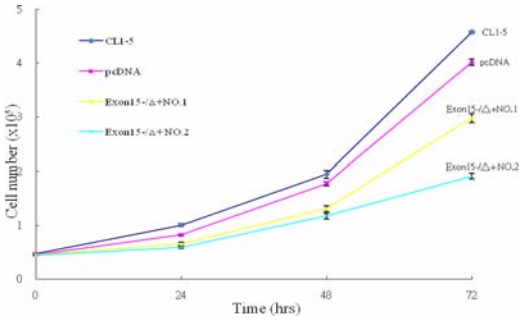


Figure 1

A. Overexpression of slit2 Exon 15-/△$^+$ reduced cell growth in CL1-5 cells.

During construction of full length slit2, we found exon 15 splicing variant in cDNA, the ratio of exon 15$^+$ to exon 15$^-$ was 1 : 7. Therefore, the majority of slit2 cDNA in cancer cell lines was exon 15$^-$. When slit2 exon 15$^-$/△$^+$ was overexpressed in CL1-5 cells, it reduced cell growth and increased doubling time about 12 hr compared to the vector control (Figure 2).



(A)

Doubling time	
	hr
CL1-5	26
pcDNA	26.3
Exon 15$^-$/△$^+$NO.1	38.2
Exon 15$^-$/△$^+$ NO.2	37.3

(B)

Figure 2. (A) Growth curve of two slit2 exon 15$^-$/△$^+$ stable clones compared with vector control and CL1-5 parental cells. (B) Doubling time of cell lines in (A).

B. Overexpression of slit2 exon $15^{-}/\triangle^{+}$ reduced invasive ability of CL1-5 cells

To investigate the role of slit2 in cell mobility and invasive ability, we performed wound healing assay and *in vitro* invasion assay. When CL1-5 cells overexpressed slit2 exon $15^{-}/\triangle^{+}$, the invasive ability of CL1-5 cells also inhibited by slit2 exon $15^{-}/\triangle^{+}$(Figure 3). This inhibition of invasive ability may be counted by both decreased in cell motility by wound healing assay (figure 4) and MMP9 expression by gelatin zymography assay (Figure 5).

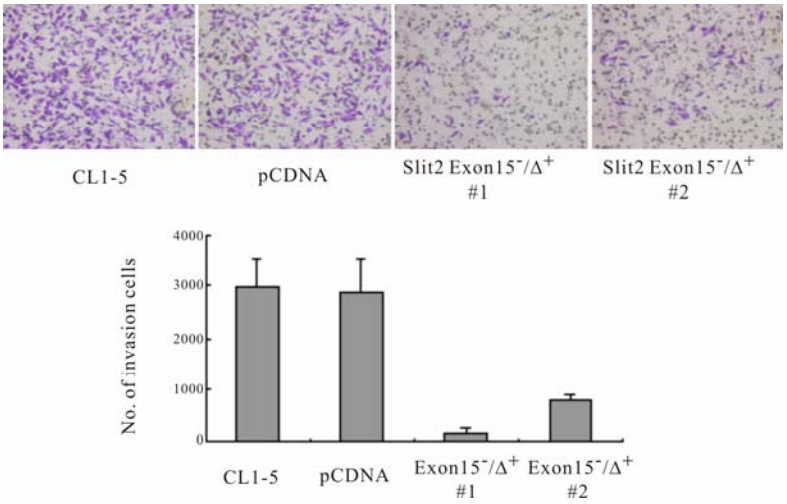


Figure 3. Overexpression of Slit2 exon $15^{-}/\Delta^{-}$ inhibited cell invasive ability of CL1-5 cell.

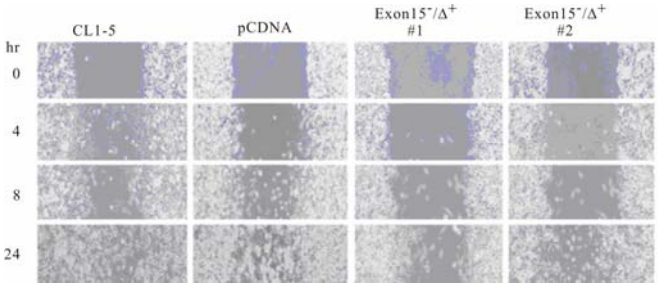


Figure 4. Wound healing assay of CL1-5 cells transfected with Slit2 exon $15^{-}/\Delta^{+}$.

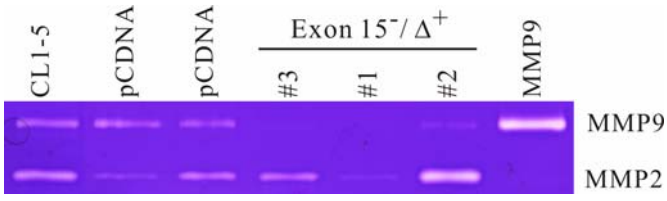
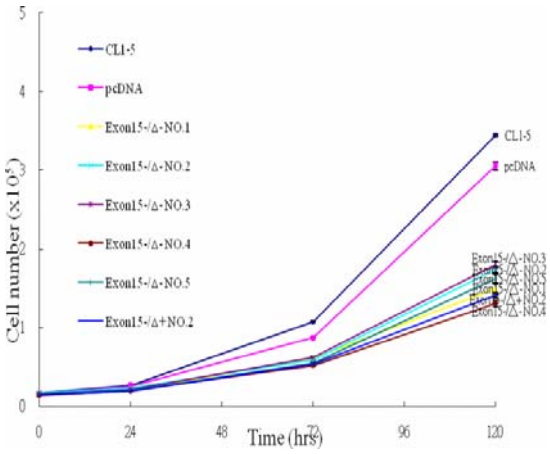


Figure 5. Gelatin Zymography assay of CL1-5 cells transfected with Slit2 Exon 15-/Δ^{+}.

C. The role of cleavage site on cell growth and invasive ability

Since Slit2 has been reported to be cleaved into 140 kD and 60 kD fragments, it is important to examined whether this cleavage is required for the growth inhibition and invasive inhibition abilities. Although we have not been able to detect any cleavage product of overexpressed slit2 exon 15-/△$^{+}$ by both anti-myc or anti-Slit2 antibody, we still overexpressed slit2 exon 15-/△$^{-}$ in CL1-5 for investigating the role of cleavage in Slit2 function. Our results showed that without cleavage site, Slit2 still has ability to inhibit cell growth (Figure 6) and mobility (Figure 7).



(A)

Doubling time	
	hr
CL1-5	25.4
pcDNA	28.1
exon 15-/△$^{-}$ NO.1	40.2
exon 15-/△$^{-}$ NO.2	40.1
exon 15-/△$^{-}$ NO.3	36.4
exon 15-/△$^{-}$ NO.4	40.5
exon 15-/△$^{-}$ NO.5	37.4

(B)

Figure 6. (A) Growth curve of two slit2 exon 15^{-}/△$^{-}$ stable clones compared with vector control and CL1-5 parental cells. (B) Doubling time of cell lines in (A).

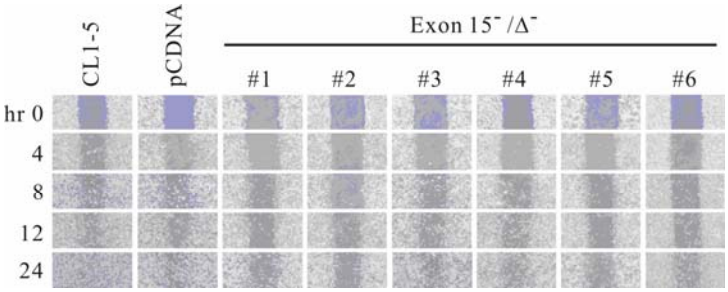
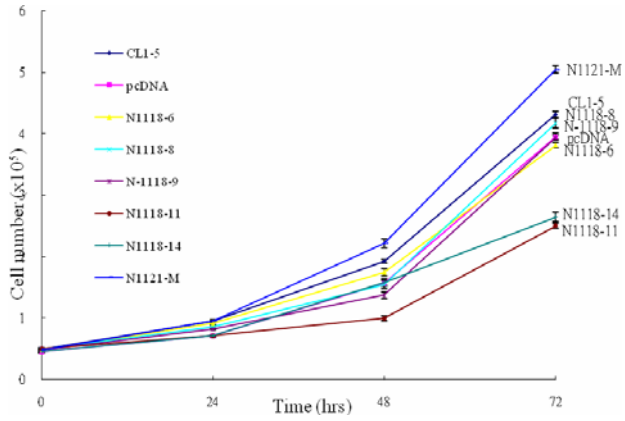


Figure 7. Wound healing assay of slit2 exon 15-/△$^{-}$ in CL1-5 cells.

D. The role of slit2 N- and C- terminal fragments on cell growth and invasion

To identity functional domain of Slit2 that involved in growth inhibition and invasive inhibition, Slit2 N-terminal and C-terminal fragment was generated based on the reported cleavage site. When Slit2 C-terminal was stably expressed in CL1-5, the growth rate and mobility of CL1-5 were both inhibited, while Slit2 N-terminal did not affect cell growth (Figure 8) or mobility (Figure 9). These results were rather surprising, since Slit2 N-terminal

has been implicated to be responsible for inhibition of neuron cell migration via interaction with Robo1. However, the N-terminal used in this experiment did not contain the sequence of exon 15. We were wondering whether exon 15 may play an important role in Slit2 function.



(A)

Doubling time	
Hrs	0~24
CL1-5	26
pcDNA	26.3
N1118-6	24.3
N1118-8	28.3
N1118-9	25.3
N1118-11	28.3
N1118-14	33.9

(B)

Figure 8. (A) Growth curve of two slit2 N1118 stable clones compared with vector control and CL1-5 parental cells. (B) Doubling time of cell lines in (A).

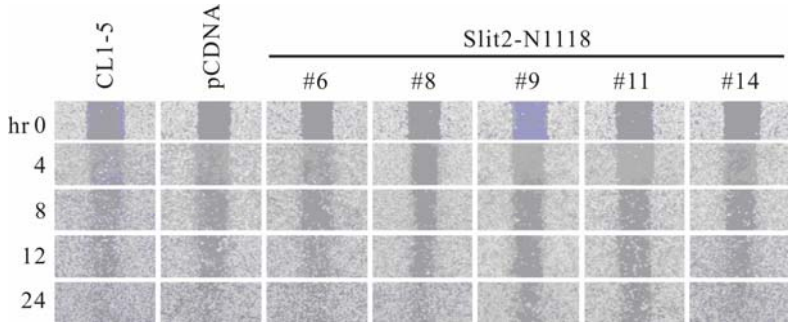
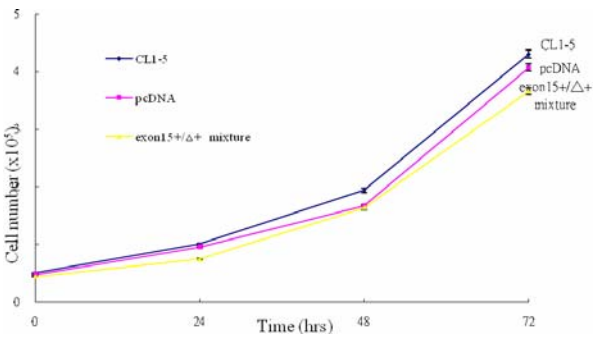


Figure 9. Wound healing assay of Slit2-N1118 overexpressed in CL1-5 cells.

E. The role of Exon 15 on cell growth and migration

To clarify the role of exon 15 in Slit2 function, the Slit2 exon $15^{+}/\triangle^{+}$ was overexpressed in CL1-5. Unexpectedly, exon 15 showed different effect on Slit2 function in that Slit2 exon $15^{+}/\triangle^{+}$ did not decrease cell growth as Slit2 exon 15-/$\triangle^{+}$ did in CL1-5 (Figure 10), whereas Slit2 exon $15^{+}/\triangle^{+}$ still be able to block cell mobility (Figure 11). This result suggests that exon 15 may modulate inhibitory roles of Slit2 in cell growth but not in motility.

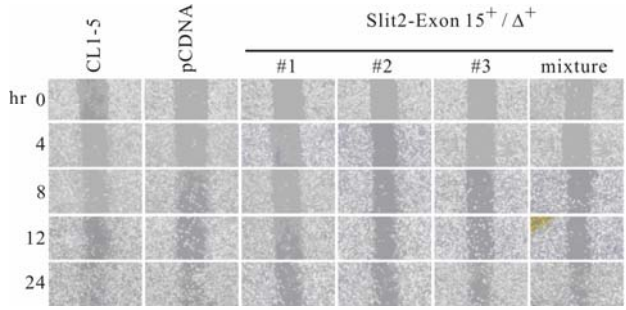


(A)

Doubling time	
	hr
CL1-5	25.7
PcDNA	25.6
exon 15+/△+ NO.1	26.0
exon 15+/△+ NO.2	27.9
exon 15+/△+ mixture	28.4

(B)

Figure 10. (A) Growth curve of Slit2 exon $15^{+}/\triangle^{+}$ stable clone mixture compared with vector control and CL1-5 parental cells. (B) Doubling time of cell lines in (A).



Figrue 11. Wound healing assay of Slit2 exon $15^{+}/\triangle^{+}$ overexpressed CL1-5 cells.

F. The role of Slit2 in foci formation assay and anchorage independent growth

Both Slit2 exon 15-/$\triangle^{+}$ and Slit2 C-terminal fragment inhibited CL1-5 cell growth, and reduced colony size in foci formation assay, while Slit2 N- terminal fragment formed similar sizes of colony as vector control (Figure 12). Similarly, Slit2 C-terminal but not N-terminal fragment reduced colony formation in anchorage independent growth (Figure 13).

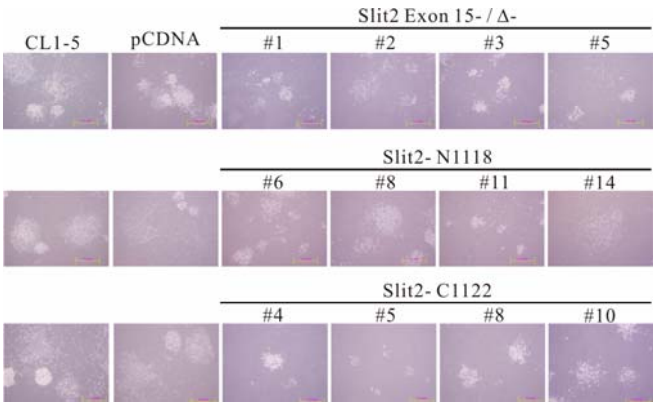


Figure 12. Foci formation assay of CL1-5 cells expressing various Slit2 fragments.

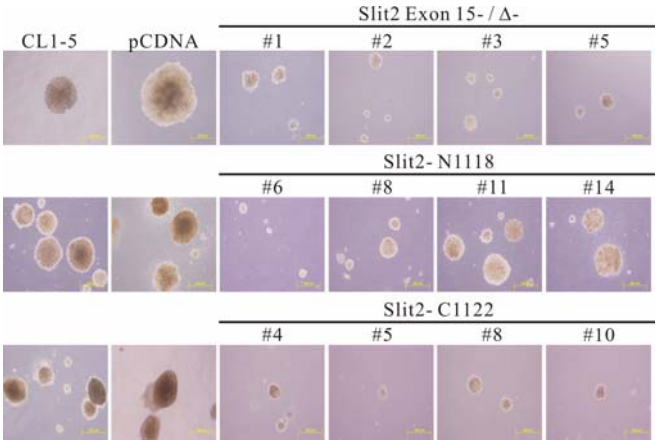


Figure 13. Anchorage independent assay of CL1-5 cells expressing various Slit2 fragments.

G. Overexpression of Slit2 Exon 15-/△$^{+}$ and Slit2 C-terminal increased G0/G1 phase cell population

To investigate which phase of cell cycle would be affected by Slit2 induced cell growth inhibition, CL1-5 cells overexpressed with different Slit2 fragments were analyzed by flow cytometry. Our results showed that similar to Slit2 Exon 15-/△$^{+}$, Slit2 C-terminal increased G0/G1 phase cell population while Slit2 N-terminal has the same cell cycle distribution as vector control (Figure 14).

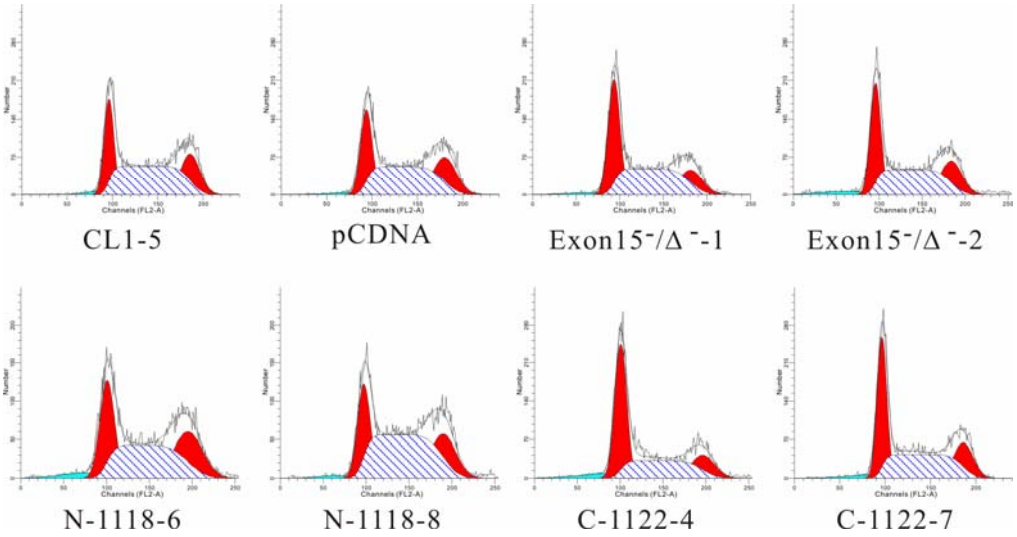


Figure 14. Cell cycle distribution of CL1-5 cells expressing various Slit2 fragments analyzed by flow cytometry.

五、討論:

Slit2 plays important role in neural development. Its prominent biological role is in the area of axon migration and migration of various cell types. Recently, the pathological role of slit2 has been revealed in many cancers. We found that the expression of slit2 is highly repressed in lung cancers. While the expression of slit2 is very low in most of lung cancer cell lines, its expression pattern is quite unique in CL1 series in that the expression of slit2 is inversely correlated with the invasive ability. This phenomenon prompted us to investigate whether repressing of Slit2 expression would enhance cancer invasion. To test this hypothesis, we overexpressed slit2 in high invasive cell line, CL1-5. Indeed, Slit2 highly repressed cell invasive ability in CL1-5 (figure 3). This inhibitory effect may be a combination of reduction of mobility and MMP9 expression. In addition to inhibitory effect of invasion, we found that Slit2 inhibited cell growth.

It has been reported that Slit2 protein can be cleaved extracellularly into a 140 kd and 55-60 kd fragments. The N terminal fragment possessed axonal repellent role as the full length protein, however the biological role of C terminal fragment remains unclear. Since we have not been able to detect the cleavage product of the exogenous Slit2-myc fusion protein in CL1-5 cells, we were wondering whether the cleavage event is required for the inhibitory roles of Slit2 in invasion and growth. To test this, a cleavage site negative full-length Slit2 was created and expressed in CL1-5 cell. Our result showed that in the absence of cleavage site, Slit2 still possessed inhibitory roles in invasion and growth. To further dissect the domains of Slit2 that confer inhibitory role of invasion and/or growth, we generate CL1-5 cell stably expressed Slit2-N terminal or C-terminal fragments. The results showed that Slit2- C terminal fragment itself has potential to inhibit cell growth and migration. One of our important goals is to identify molecule that would transducer Slit2- C terminal signal.

In the process of cloning the full-length slit2, we identified exon 15 splicing variants. The ratio of cDNA containing exon 15- and exon 15^+ was 7 to 1. It is of interest to investigate whether exon 15 plays a role in Slit2 function. When full-length Slit2 containing exon 15 (Slit2-exon $15^+/\Delta^+$) was expressed in CL1-5, it still possessed the ability to inhibit mobility, however the inhibitory role in cell growth was lost. The exon 15 is located at the end of the second leucin rich repeat (LRR). The axon repellent role of Slit2 is mediated by direct interaction of second LLR to Robo 1 receptor. It is not known the role of exon 15 in Slit2-Robo 1 interaction. It is possible that the LRR structure may be influenced by exon 15 and that in turns affect its ability to bind Robo1 or other Robo families. Alternatively, exon 15 may interact yet unknown receptor that would interfere with the interaction between LRR and Robo. It is still not known whether the role of growth inhibition would be mediated by Robo receptor. The RNAi will be used to investigate the role of Robo in Slit2 mediated invasion and cell growth inhibition.

六、參考文獻:

Chang J.T., Chang H., Chen P.-H., Lin S.-L., and Lin P. (2007) Requirement of AhR overexpression for CYP1B1 up-regulation and cell growth in human lung adenocarcinomas. Clinical Cancer Res. 13 (1):38-45

劉智雄(2008)”構築不可切割之 Slit2 蛋白基因於 pcDNA 載體”
中山醫學大學醫學分子毒理學研究所碩士論文

莊書銘(2008)”探討 Slit2 蛋白片段對人類肺癌細胞株 CL1-5 生長與遷徙之影響
中山醫學大學醫學分子毒理學研究所碩士論文

七、計畫成果自評:

Our study has revealed unexpected roles of Slit2 in lung carcinogenesis. Down regulation of Slit2 showed inhibition of cell growth and invasion. Our future works will focus on several fields as follows: (1) Narrow down the effect domain of Slit2- C terminal fragment that would reduce cell growth and inhibit motility. (2) Study how exon 15 affect Slit2-meidated cell growth inhibition. (3) Identify pathways that involved in biologic effects conferred by various Slit2 fragments. (4) Study the effect of Slit2 in xenograft model.

This was original a three-year project but was funded as a two-year project. We had finished most part of the first year and the third year project. The project involved RNAi is still underway. The goal to pursue down stream target genes via microarray analysis is still pending.

If we are able to map small peptide fragments of Slit2 that would mediate cell growth inhibition and invasion inhibition, these peptides may be used as therapeutic agent for lung cancer. Hopefully our study of slit2 in lung cancer would provide good insight of slit2 in tumorigenesis and even its function in normal lung. I am confident that our study would be valuable for further understanding lung carcinogenesis and should be publishable.