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Study the signaling pathway of slit2 invovled in lung cancer cell growth and invasion(第 2 年)
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計 畫 主 持 人：蔡菁華

計畫參與人員：碩士級-專任助理人員：孫仕容
碩士班研究生-兼任助理人員：林毓瑩
碩士班研究生-兼任助理人員：劉思妤
碩士班研究生-兼任助理人員：邱君玲
碩士班研究生-兼任助理人員：陳美妤
碩士班研究生-兼任助理人員：王吉祥

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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

Study the signaling pathway of Slit2 involved in lung cancer cell growth and invasion

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共同主持人：
計畫參與人員： 林毓瑩、劉仲秋、張彗怡、孫仕容、邱君玲、劉思妤、王吉祥、陳美妤

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中文摘要

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。有報告指出Slit2具有抑制癌細胞生長、侵襲的功能，相反的也有研究指出Slit2具有促進血管新生的功能，進而促進腫瘤的生長。因此，Slit2在癌症的發展上所扮演的角色仍有待釐清。我們發現Slit2在exon 15的位置上有剪接變異的表現。不具有exon 15的Slit2，稱為Slit2-ΔE15，具有抑制肺癌細胞CL1-5的生長及侵襲能力。而具有exon 15的Slit2，稱為Slit2-WT，則只具有抑制細胞侵襲的能力。過去的研究指出Slit2透過Robo1受體抑制神經細胞軸突及其他細胞的移動能力。欲了解Slit2-ΔE15及Slit2-WT是否透過不同的Robo受體執行不同的功能，我們利用RNA干擾技術將在CL1-5細胞有表現的Robo1及Robo4分別給予抑制。結果發現，抑制CL1-5細胞的Robo4表現後，Slit2-WT失去了抑制細胞侵襲的能力。但是，抑制Robo1的表現並不影響Slit2-WT抑制細胞侵襲的能力，因此確定Slit2透過Robo4而非Robo1抑制細胞侵襲的能力。有趣的是，Slit2-ΔE15抑制細胞侵襲能力則不需要Robo1及Robo4這兩個受體，因為抑制這兩個受體的表現後，Slit2-ΔE15仍然有效的抑制細胞的侵襲能力。雖然，Robo1及Robo4在Slit2-ΔE15抑制細胞侵襲能力上不扮演任何角色，抑制這兩個受體後會降低Slit2-ΔE15抑制CL1-5細胞生長的能力。因此，Robo1及Robo4都參與了Slit2-ΔE15抑制細胞生長的途徑。另一方面，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。沒有exon 15的Slit2-N端片段(Slit2-N-ΔE15)也具有抑制生長的能力而有exon 15的Slit2-N端片段(Slit2-N-WT)則不會抑制生長。但是，無論是Slit2-N-ΔE15或是Slit2-N-WT都不具有抑制細胞的侵襲能力。出乎意料的是Slit2的C-terminal片段本身即具有抑制細胞侵犯及生長的能力。我們的研究有兩個重要的發現: (1) exon 15的存在與否可以決定Slit2抑制生長的能力，這可能是因為exon 15存在與否將改變Slit2與不同的受體的親和力進而影響其功能 (2)本研究顯示了不為人知的Slit2 C-terminal片段在抑制細胞的生長與侵犯上的角色。

英文摘要

Slit2 is a secreted glycoprotein which was first identified as a repellent molecule of axon guidance. Most biological functions of Slit2 are referred to its ability of affecting cell migration in various cell types. Recently, more evidences exhibited extra functions of Slit2 in carcinogenesis and angiogenesis. Slit2 is highly expressed in normal lung, but greatly repressed in lung cancer. We identified exon 15 splicing variants in CL1-0 lung cancer cells as well as non-tumor lung tissue. In the absence of exon 15, Slit2-ΔE15 represses growth and invasion of CL1-5 lung cancer cells. However, with the exon 15, Slit2-WT only suppresses cell invasion. Previous studies pointed out that Robo1 is involved in Slit2-mediated axon and cell migration. Since CL1-5 cells only express Robo1 and Robo4, we performed RNA interference to knock-down the expression of Robo1 or Robo4 to explore whether Slit2-ΔE15 and Slit2-WT utilize different Robo receptors to execute their functions. Our results showed that knock-down Robo4 but not Robo1 restores the invasive capability of CL1-5 overexpressing Slit-WT. However, neither Robo1 nor Robo4 is involved in Slit2-ΔE15-mediated invasion inhibition in CL1-5 cells, indicating that other receptor(s) is/are involved in Slit2-ΔE15-mediated invasion inhibition. Although Slit2-ΔE15-mediated invasion inhibition does not require Robo1 and Robo4, both of the receptors are involved in Slit2-ΔE15-mediated growth inhibition of CL1-5 cells. It has been shown that Slit2 protein can be cleaved into two fragments: a 140 kD Slit2-N and a 55-60 kDa Slit2-C fragments, and the N-terminal Slit2 is sufficient to inhibit neuron cell migration. We generated CL1-5 stable clones expressing Slit2-N-ΔE15, Slit2-N-WT and Slit2-C, respectively. Our results showed that similar to the effect of full-length Slit2 on growth inhibition, Slit2-N-ΔE15 but not Slit2-N-WT represses cell growth. Contrary to previous studies reported Slit2-N function, we found that both Slit2-N-ΔE15 and Slit2-N-WT failed to inhibit cell invasion of lung cancer cells. To our surprise, Slit2-C alone possesses both growth and invasion inhibition ability. Therefore, our studies have two important findings (1) Presence of exon 15 or not determines the ability of Slit2 in growth inhibition of lung cancer cells. The possible mechanism is that exon 15 may alter the affinity of Slit2 to different receptors that in tern change its functions. (2) Unravel an unknown function of Slit2-C terminal in growth and invasion inhibition of lung cancer cells.

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 up-rises 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 guiding axon migration. It is highly expressed in normal lung, but repressed in lung cancer (figure 1). We identified exon 15 splicing variants of slit2 in CL1-0 low invasive lung cancer cells. In the absence of exon 15, Slit2-ΔE15 represses growth and invasion of CL1-5 lung cancer cells. With exon 15, Slit2-WT only inhibited cell invasion but not cell growth. In this study, we aim to identify the receptor(s) that are involved in Slit2-ΔE15-mediated invasion and growth inhibition and Slit2-WT-meidated cell invasion. In addition, we try to dissect the domains of Slit2 that are involved in growth and invasion inhibition in lung cancer cells.

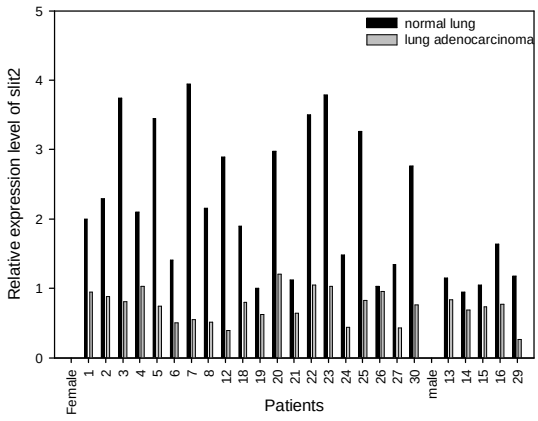

Figure 1: Relative expression level of slit2 in Taiwanese lung adnocarcinoma analyzed by microarray. Gray bars represent slit2 expression in lung adenocarcinoma, and the black bars represent normal counterpart of the same patient.

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(Kidd et al., 1999)*. 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 (Sabatier et al., 2004). 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 (Xu et al., 2004) and dendritic cell migration thus slit2 also functioning as an anti-inflammatory factor for initiating immune responses (Guan et al., 2003). 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 (Kramer et al., 2001; Wang et al., 2003). Interestingly, during myogenesis slit functions switches from repulsion to attraction. Moreover, a recent study revealed that Slit-Robo plays a role in angiogenesis (Wang et al., 2003). 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 (Astuti et al., 2004; Dallol et al., 2002; Dallol et al., 2003; Lin et al., 2007; Narayan et al., 2006). Slit2 has also been demonstrated to possess tumor suppressor activity (Dallol et al., 2002). Recently, inhibition of Slit2-Robo pathway regulates E-cadeherin expression and is associated with poorer survival in non-small cell lung cancer patients (Tseng et al., 2010).

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 (Brose et al., 1999; Rothberg et al., 1990). 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 (Brose et al., 1999). 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 (Nguyen Ba-Charvet et al., 2001). The N-terminal fragment of Slit2 has an ability to induce sensory axon elongation and branching (Wang et al., 1999).

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:

A. Overexpression of slit2-N-DE15 reduced cell growth in CL1-5 cells .

Our previous studies showed that Slit2-ΔE15 inhibited both cell growth and invasive ability, while Slit2-WT only possessed invasive inhibition ability but not growth inhibition. Further information showed that Slit2-C exhibited both growth and invasive ability while Slit2-N-WT inhibited neither growth nor invasion of CL1-5 cells. Since exon 15 plays important role in growth inhibition of full-length Slit2, we wondered if Slit2-N-ΔE15 would have growth inhibition capability. Our results showed that Slit2-N-ΔE15 still possessed growth inhibition (Table 1 & Figure 2). Therefore, both Slit2-C and Slit2-N-ΔE15 have ability to inhibit cell growth.

Doubling time	hr
CL1-5	28.5 ± 2.7
vector	26.9 ± 3.3
N^--4	32.4 ± 3.7
N^--5	55.3 ± 2.9
N^--6	59.1 ± 4.0
N^--8	69.8 ± 9.3

(A)

Doubling time	hr
CL1-5	25.8 ± 0.3
vector	27.3 ± 1.4
N^+-6	25.0 ± 0.9
N^+-8	27.5 ± 1.1
N^+-11	26.9 ± 2.2
N^+-14	32.8 ± 1.5

(B)

Table 1. Doubling time of CL1-5 cells expressed Slit2-ΔE15 and Slit2-WT.

(A) Slit2-N-DE15 (N^-) (B) Slit2-N-WT (N^+)

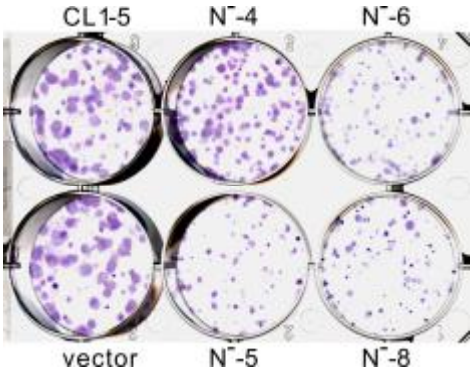

Figure 2. Adhesive growth of various CL1-5 clones stably express Slit2-N-ΔE15 (N^-).

B. Overexpression of slit2-N-DE15 did not affect invasive ability of CL1-5 cells

In neuron cell, the N-terminal fragment of Slit2 was sufficient to suppress cell migration. Our previous result showed that Slit2-C exhibited inhibition of cell invasion while Slit2-N-WT did not. To clarify if Slit2-N terminal fragment plays a role in invasion inhibition, we measured invasion ability of Slit2-N-ΔE15 in CL1-5. Our results showed that overexpression of Slit2-N-ΔE15 did not inhibit invasive ability in CL1-5 cells (Figure 3). This result showed that the invasive ability is truly possessed by C-terminal fragment of Slit2.

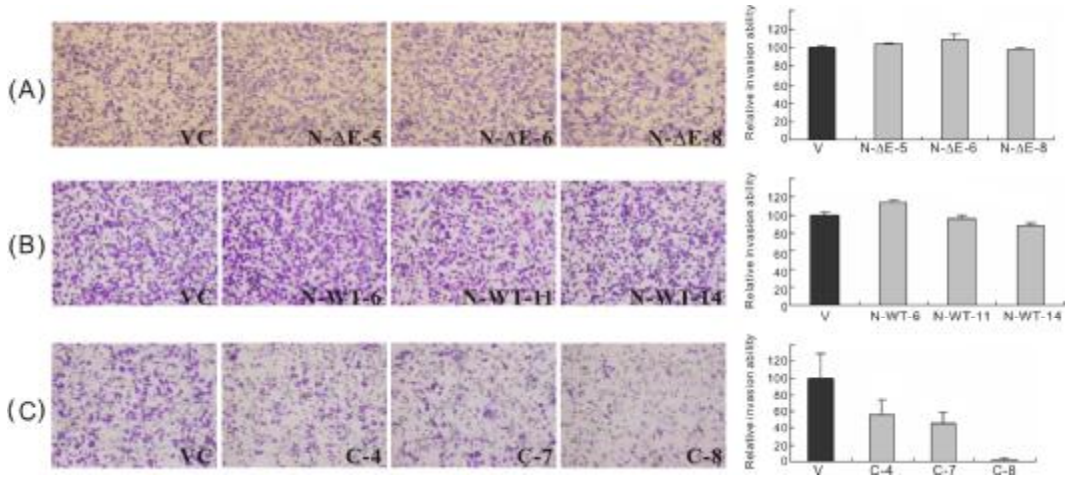

Figure 3. The effect of Slit2-N and Slit2-C terminal fragments on cell invasion. (A) Invasion ability of CL1-5/Slit2-N-ΔE15 stable clones and CL1-5/VC (B) Invasion ability of CL1-5/Slit2-WT stable clones (C) Invasion ability of CL1-5/Slit2-C

C. The effect of Slit2 containing conditioned medium on cell growth.

Since Slit2 is a secreted protein, cells treated with conditioned medium expressing various slit2 constructs ought to behave similar to cells with over-expressed Slit2. Indeed, the conditioned medium containing Slit2-ΔE15 reduced cell growth compared with one containing Slit2-WT or vector control (Figure 4).

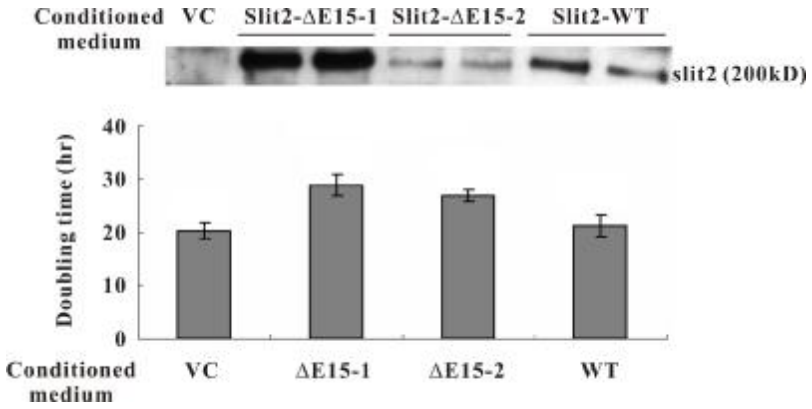

Figure 4. The effect of conditioned medium containing Slit2-ΔE15 or Slit2-WT on cell growth.

D. The effect of Slit2 containing conditioned medium on cell invasion.

Similarly, we used conditioned medium containing Slit2-ΔE15 for invasion assay and found that both of them have ability to repress invasive ability (Figure 5).

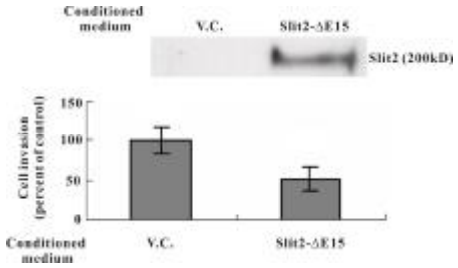

Figure 5. The conditioned medium collected from CL1-5 cells overexpressing Slit2-ΔE15 inhibits cell invasion.

E. The role of Robos in Slit2-DE15-mediated cell growth inhibition

Robo receptors have been shown to mediate Slit2 functions in cell migration and inflammatory responses. Both Robo1 and Robo4 are expressed in CL1-5 cells. To examine if Robos involved in Slit2-ΔE15-mediated invasion inhibition, we used robo1 and robo4 siRNA to explore their roles in Slit2-mediated cell growth inhibition. Our results showed that blocking either Robo1 or Robo4 expression restored the cell growth of CL1-5/slit2-ΔE15 cells (Figure 6). This data indicated that both Robo1 and Robo4 are involved in Slit2-ΔE15-mediated growth

inhibition.

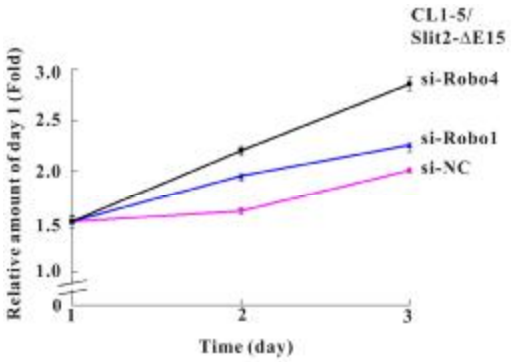

Figure 6. Knock-down expression of Robo1 or Robo4 by RNAi blocks Slit2-ΔE15-mediated growth inhibition in CL1-5 cells.

F. The role of Robos in Slit2-mediated invasion inhibition.

Evidence from previous studies has shown that Robo1 is responsible for Slit2-mediated cell migration in glioma. However, knockdown of Robo1 expression did not abolish Slit2-ΔE15- or Slit2-WT-mediated cell invasion in CL1-5 cell lines (Figure 7A). To our surprise, knockdown of Robo4 expression abolished Slit2-WT-mediated cell invasion but not Slit2-ΔE15-mediated cell invasion (Figure 7A). To confirm the importance of Robo4 in Slit2-WT-mediated cell invasion, a second si-*robo4* and anti-Robo4 antibody were applied to two independent Slit2-WT stable clones. Our results revealed that both si-*robo4-2* and anti-Robo4 restored the invasive ability of CL1-5/Slit2-WT cells (Figure 7B). These data demonstrated that Robo1 plays no role in Slit2-mediated invasion inhibition in CL1-5 cells. Instead, in the presence of exon 15, Slit2 would inhibit cell invasion through Robo4. Our results indicated that receptor(s) other than the Robo family receptors may transduce invasion inhibition signals from Slit2-ΔE15; however, this remains to be elucidated.

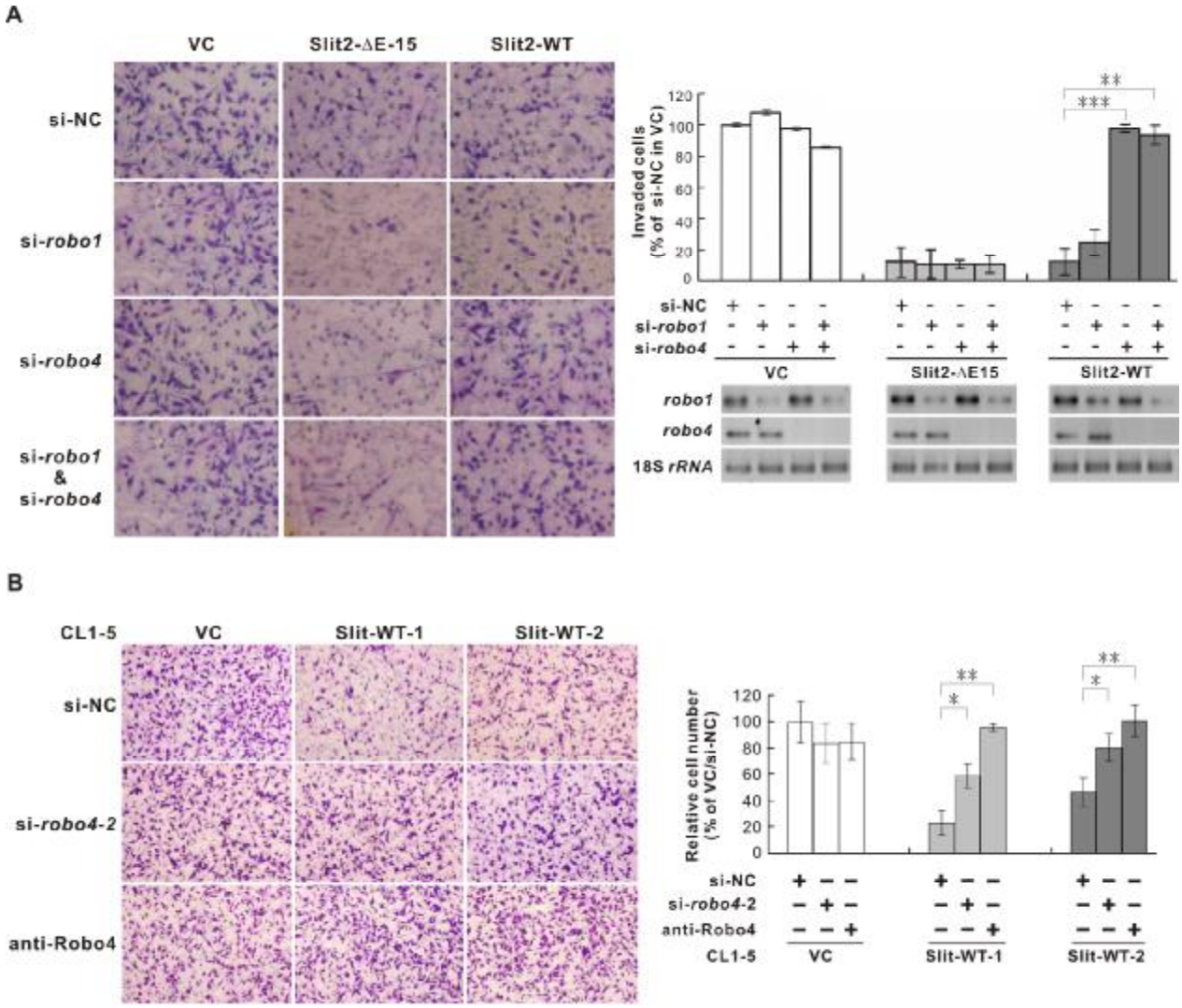

Figure 7. Robo4 is involved in Slit2-WT- but not Slit2-ΔE15-mediated cell invasion.
(A) si-Robo1 or si-Robo2/si-Robo4 did not affect Slit2-DE15-mediated cell invasion. However, si-Robo4 but not si-Robo4 restored invasion capability of CL1-5/Slit2-WT cells. (B) A second si-Robo4 and anti-Robo4 restored two independent CL1-5/Slit2-WT cells.

G. Generate small deletion constructs of Slit2 to map the domain(s) that responsible for growth or/and invasive inhibition.

Slit2 contains four leucin rich repeat (LRR), nine EGF-like repeats, a laminin G domain, and a C-terminal cycteine-rich knot (CT). A known cleavage site resides between the fifth and the sixth EFG domain, which was used to define the boundary of Slit2-N and Slit2-C constructs. The exon 15 is located at the end of second LRR. It has not been reported that the region of exon 15 involved in the interaction between Slit2 and Robo1. Since presence of exon 15 inhibited growth inhibition ability of Slit2, it is possible that presence of Slit2 might forms a structure which is unfavorable for Slit2 binding to a receptor that confers growth inhibition or alternatively, having

or not exon 15 could lead Slit2 to different binding proteins. To further mapping domain(s) that may involved in growth or/and invasive inhibition, we deleted each LRR domain, 1-6 EFG, 7-9 EFG domain with laminin G domain, and CT domain. We have identified two distinct domains in Slit2-C terminal fragment that play important role in growth and invasion inhibition respectively.

H. Xenograft model

To determine whether Slit2-ΔE15 and Slit2-WT display different biological functions, we injected CL1-5/Slit2-ΔE15, CL1-5/Slit2-WT or CL1-5/VC cells subcutaneously and dorsally into immunodeficient mice. We observed that CL1-5/Slit2-ΔE15 cells formed smaller tumors when compared with the tumors formed by CL1-5/Slit2-WT and CL1-5/VC cells. Growth of tumors formed by Slit2-WT-overexpressing CL1-5 cells and by vector control cells was comparable (Figure 8A). The CL1-5 cells harboring empty vectors formed firm tumors that invaded the front limbs and ribs of the mice. In contrast, CL1-5 cells expressing exogenous Slit2 formed soft, hemorrhagic, and necrotic tumors. Interestingly, the number and size of metastastic nodules in the liver and lung decreased significantly in the cells expressing exogenous slit2-WT when compared with the vector control cells (Figure 8B). This xenografted screening strongly suggested that Slit2-ΔE15 and Slit2-WT have distinct roles in tumor growth.

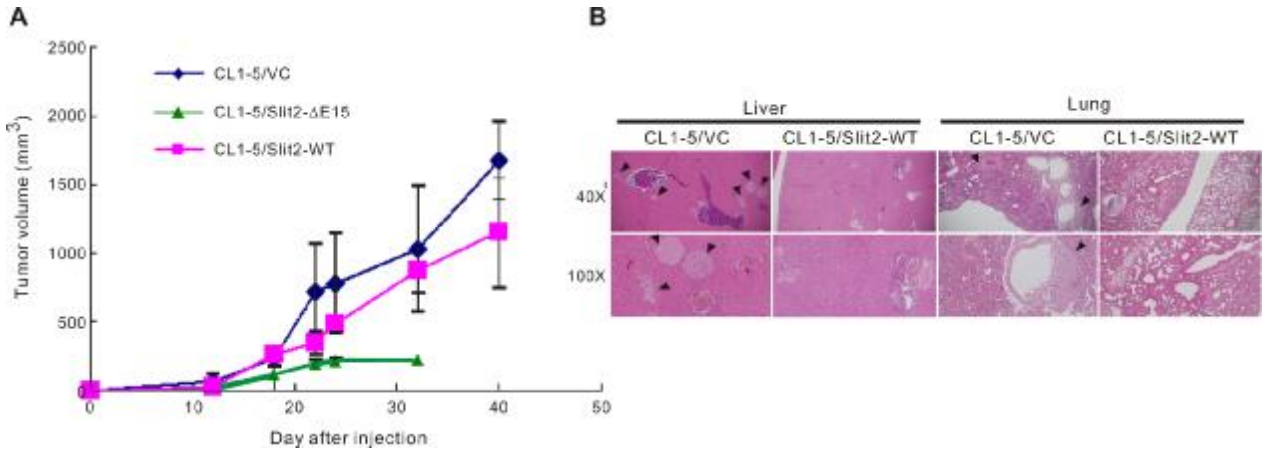

Figure 8. The growth and metastasis of CL1-5/Slit2-ΔE15 and /Slit2-WT in Xenograft model

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. 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.

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. In the absence of exon 15, Slit2-ΔE15 represses both growth and invasion of CL1-5 lung cancer cells. When full-length Slit2 containing exon 15 (Slit2-WT) was expressed in CL1-5, it still possesses the ability to inhibit invasion ability, however the inhibitory role in cell growth was lost. The conditioned medium containing Slit2-WT or Slit2-ΔE15 conferred the same effect as seen in over-expression cell lines. The axon repellent role of Slit2 was reported to be mediated by direct interaction of second leucin rich repeat (LRR) to Robo 1 receptor. The exon 15 is located at the end of the second LRR of Slit2. We speculated that exon 15 may influence LRR2 structure and change the binding affinity of Slit2 to its Robo receptors. Both Robo1 and Robo4 are expressed in CL1-5 cells. Thus, we performed RNA interference to explore if Slit2-ΔE15 and Slit2-WT execute their function through different Robo receptors. Our findings showed that both Robo1 and Robo4 are required for Slit2-ΔE15-mediated growth inhibition. Two possible mechanisms may explain how Robo1 and Robo4 are involved in Slit2-ΔE15-mediated growth inhibition. First, Slit2-ΔE15 may bind to Robo1 or Robo4 independently and transduce growth inhibitory signals. Secondly, Slit2-ΔE15 may enhance Robo1/Robo4 heterodimer formation for initiating growth inhibition activity.

By contrast, regardless of the exon 15 status, both Slit2-ΔE15 and Slit2-WT conferred the ability to inhibit cell invasion. It has been shown that the Slit2/Robo1 interaction transduces a signal that navigates the growth of olfactory bulb axons in *Drosophila* and inhibits glioma cell migration/invasion (Mertsch et al., 2008; Yiin et al., 2009). Intriguingly, our results showed that Slit2-mediated invasive inhibition activity in CL1-5 cells was not transduced by Robo1, since knockdown of Robo1 expression did not restore the invasive ability of CL1-5 cells expressing ectopic Slit2-ΔE15 or Slit2-WT. Surprisingly, the status of exon 15 seems to determine pathways that are involved in the invasive inhibition of Slit2 as a knockdown expression of Robo4 abolished Slit2-WT-mediated invasive inhibition but not Slit2-ΔE15-mediated invasive inhibition.

Biochemical studies revealed that LRR2 of *Drosophila* Slit interacts with Robo1, 2, and 3 receptors (Howitt et al., 2004), and crystal structure study further confirmed direct interaction between LRR2 of human Slit2 and the first 2 Ig domains of Robo1 (Morlot et al., 2007). However, the region of LRR2 (residues 271 to 479) used in the crystal structure study did not contain exon 15 (residues 480 to 487). Thus, it is possible that the presence of exon 15 would alter Slit2 structure, resulting in changed affinities to the various Robo receptors. We hypothesize that in the presence of exon 15, Slit2-WT will bind to Robo4 instead of Robo1. The Robo4 monomer or Robo4 homodimer would then transduce the invasive inhibition signal but not the growth inhibition signal. Therefore, it is possible that presence of exon 15 would form unfavorable structure for interaction between LRR2 and Robo1 but favor the interaction between Slit2 and Robo4.

Our results implicate that exon 15 might modulate the binding affinity of Slit2 to its various receptors and thus influence its role in growth and invasion in CL1-5 lung cancer cell lines. Our current model is that in the absence of exon 15, Slit2-ΔE15 binds to Robo1 and subsequently interacts with Robo4 to recruit adaptor proteins for growth inhibition. In the presence of exon 15, Slit2 binds to Robo4 but not to Robo1 that would recruit another set of adaptors to execute the invasion inhibition function. Our study reveals complex Slit2 signaling pathways and implicates existing alternate pathway(s) that would transduce Slit2-ΔE15-mediated cell invasion.

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 generated 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. We are doing smaller deletion in slit2 to narrow down domain requires for growth or invasive inhibition.

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七、計畫成果自評:

Our study has revealed unexpected roles of Slit2 in lung cancer growth and invasion. Up regulation of Slit2-ΔE15 showed inhibition of cell growth and invasion while overexpression of Slit2-WT only represses cell invasion. The status of exon 15 in Slit2 determines the pathways involved in their functions. Our future works will focus on several fields as follows: (1) Identify receptor(s) involved in Slit2-ΔE15-meidated invasion inhibition. (2) Identify pathways involved in Slit2-WT and Slit2-ΔE15 functions. (3) Identify receptor(s) involved in Slit2-C mediated growth and invasion inhibition. (4) Narrow down the effect domain of Slit2- C terminal fragment that would reduce cell growth and inhibit motility.

We are hoping to map small peptide fragments of Slit2 that would mediate cell growth inhibition and/or invasion inhibition, these peptides may be used as therapeutic agent for lung cancer treatment. Hopefully our study of slit2 in lung cancer would provide good insight into slit2 function 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.

無衍生研發成果推廣資料

97 年度專題研究計畫研究成果彙整表

計畫主持人：蔡菁華	計畫編號：97-2320-B-040-012-MY2
計畫名稱：Study the signaling pathway of slit2 invovled in lung cancer cell growth and invasion	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	2	100%	篇	已發表之論文雖非本計劃相關之課題，但使用計畫經費完成，另一篇本計劃論文已在審查中，一篇撰寫中。
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
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	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	7	0	100%	人次	
		博士生	1	0	100%		
		博士後研究員	0	0	100%		
		專任助理	1	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 ■未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

已有一篇論文正在 Cancer 期刊審查，另一篇論文將要投稿。

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限）

本研究發現 Slit2 蛋白在肺癌組織中的表現有被抑制的現象，我們進而發現正常肺組織中 Slit2 有剪接變異型的表現，而此 Slit2 剪接變異型的表現在肺癌附近的非腫瘤組織中也可以發現。由於剪接變異型的蛋白經常具有不同的功能，因此，我們將 Slit2 的兩種剪接變異型的蛋白表現於具有高度侵犯能力的 CL1-5 肺癌細胞株中探討它們的功能。我們發現其中一種變異型的蛋白具有抑制肺癌細胞的生長及侵襲能力，而另一種則只有抑制侵襲的能力。利用 RNA 干擾技術，我們更進一步發現這兩種剪接變異型的蛋白會透過不同的受體執行其抗腫瘤的功能。因此，我們期望本研究最後能確立不同剪接變異型的 Slit2 抑制細胞生長及侵襲能力的訊息傳遞路徑。另外，我們正在確定 Slit2 蛋白中具有抑制生長及侵襲能力的最小區域，希望這些小的胜肽片段將來可以用來抑制腫瘤的生長或者轉移的能力。