行政院國家科學委員會專題研究計畫　期中進度報告

分析肺臟轉殖胎盤生長因子小鼠的肺臟發育(1/2)

計畫類別：個別型計畫
計畫編號：NSC92-2314-B-002-222-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：國立臺灣大學醫學院小兒科

計畫主持人：曹伯年
共同主持人：李鴻，謝豐舟
計畫參與人員：莊麗瑛

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 93 年 5 月 12 日

附件一

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

(計畫名稱)

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計畫主持人：曹伯年
共同主持人：謝豐舟、李　鴻
計畫參與人員：蘇怡寧、莊麗瑛

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：台灣大學醫學院附設醫院小兒部

中　華　民　國　93　年　5　月　5　日

中英文摘要

（一）計畫中文摘要
關鍵詞：早產兒慢性肺疾病，血管內皮細胞生長因子，血管新生蛋白，胎盤生長因子

經過廣泛使用產前類固醇，肺部表面張力素治療，以及新生兒照顧技術的進步，極低體重早產兒的存活率已大大的提高，但是早產兒慢性肺疾病卻仍然是早產兒的主要併發症之一，其發生率約在百分之 15 到 50 之間。

早產兒慢性肺疾病的原因包括早產、長期的氧氣治療、呼吸器傷害、和感染。病理變化包括肺泡發育不良、血管生長停滯、以及程度不等的纖維化。

從出生到成人階段，在肺泡的發育過程中，包括肺泡以及微血管表面積增加了約有 20 倍之多。目前對於肺泡微血管大量增加的機轉仍不清楚，只知道肺泡微血管大量增加和肺泡的形成有很大的關係。

在血管生成及血管新生的過程中，血管內皮細胞生長因子家族扮演著相當重要的角色。目前已知早產兒慢性肺疾病的病人其肺臟除了血管發育不全外，血管內皮細胞生長因子(VEGF)及其接受器(Flt-1)的表現量也明顯減少。至於，對於 PlGF，另一個 Flt-1 的受體(ligand)，在肺泡微血管的形成上所伴演的角色，目前則仍未被報告過。而在我們之前完成的 PlGF 全身過度表現的基因轉殖鼠中，我們發現它們外觀雖然看不出異常，但是它們的肺泡明顯有發育不良的現象，足見 PlGF 確實在肺臟的發育中扮演一重要角色。

為釐清 PlGF 對肺臟發育的影響，今年我們已成功的利用肺臟獨特性的 promoter，來讓 PlGF 只單純在肺臟過度表現的基因轉殖鼠。目前計畫進展順利，我們已按照計畫進度於第一年成功建立肺臟獨特過度表現胎盤生長因子的基因轉殖鼠，並已往下繁殖四個不同基因表現量的基因轉殖鼠。另外，我們亦已證實外加胎盤生長因子蛋白會促進肺臟上皮細胞死亡及抑制其增生，顯見過量的胎盤生長因子對肺臟應有不好的影響。希望在接下來一年的計畫中，能成功建立慢性肺疾病的動物模式。如此，不僅可讓我們瞭解 PlGF 直接對肺臟發育的影響，更可以利用此動物模式來探討肺泡發育的機轉，並進而找出治療早產兒慢性肺疾病的方法。

（二）計畫英文摘要

Keywords: Bronchopulmonary dysplasia, vascular endothelial growth factor, angiopoietin, placenta growth factor

After the widespread use of antenatal steroid, exogenous surfactant therapy, and improvements in neonatal care, the survival rate of very low birth weight infants has increased, but bronchopulmonary dysplasia (BPD) persists as one of the major complications in premature infants who need prolonged ventilator support. The incidence of BPD ranges between 15 and 50%.

The etiology of BPD included the immaturity, prolonged oxygen therapy, barotrauma, and infection. The pathological finding in the premature infants with BPD include alveolar hypoplasia, vascular arrest and adaptive dysmorphic changes, and variable interstitial proliferation.

During the period of alveolarization, the lung also undergoes marked vascular growth as reflected by the 20-fold increase in alveolar and capillary surface areas from birth to adulthood. Mechanisms that increase vascular surface area during late gestation and the early postnatal period are poorly understood, but it is clear that coordination of distal air space and vascular growth is essential for normal lung development.

Vascular endothelial growth factor (VEGF) family has been found to play an important role in vascuogenesis and angiogenesis. It has been well established that disrupted pulmonary vasculature and decreased VEGF and Flt-1, but no changes in Flk-1, in human infants dying with BPD. However, the role of PlGF, another ligand of Flt-1, in lung development is unknown. In our previous study, we found that overexpression of PlGF, using CMV promotor, seems disrupt pulmonary alveolarization. It give us a hint that PlGF may play an important role in lung development.

In this year, we have used lung-specific promotor, SP-C (a gift from Dr. Whitsett, University of Cincinnati), to successfully generated four different SP-C-PlGF transgenic lines with different mRNA expression levels, confirmed by real time RT-PCR. In addition, we also demonstrated that exogenous recombinant PlGF promoted type II pneumocytes cell death and inhibited cell proliferation in vitro. In the next year, we will try to demonstrate the phenotype of this transgenic mouse and setup the animal model for chronic lung disease.

報告內容

(一)、前言:

Bronchopulmonary dysplasia is a major complication in premature infants

Bronchopulmonary dysplasia was originally described by Northway as chronic lung changes in a group of 32 premature neonates who survived mechanical ventilation for treatment of respiratory distress syndrome (RDS) (1). This definition included clinical, radiological, and pathological criteria. Despite the widespread use of antenatal corticosteroids, exogenous surfactant therapy, and better clinical management of immature neonates has resulted in increased survival rates of infants who are born prematurely at less than 28 weeks gestation, with birth weights less than 1000 g, bronchopulmonary dysplasia (BPD) persists as one of the major complications in premature infants who required mechanical ventilation (2-4). Moreover, the increasing survival of very immature infants has produced an increase in the number of infants with BPD. In Taiwan, we had compared the outcome of 73 neonates born with their birth weight of 500-999 gm in National Taiwan University Hospital (NTUH) during the period between January 1, 1993 and December 31, 1996 (Period II), with the outcome of 21 such neonates born between April 1, 1988 and October 31, 1992 (Period I). The incidences of the extremely low birth weight infants increased from 23/10,173 (0.23%) in Period I to 81/13,835 (0.59%) in Periods II. The total survival rate was significantly increased in Period II (60% versus 48%), but the incidence of BPD was not decreased significantly (48% versus 58%) (5).

Impaired alveolarization in bronchopulmonary dysplasia

Bronchopulmonary dysplasia is now a disease that is seen primarily in preterm newborns who weight less than 1000 g and are born at 24-26 weeks gestation. The lung at this period is still in the canalicular stage of development and is just beginning to enter the sacular stage. The gas-exchange region of the architecturally immature lung is composed of large structures, referred to as saccules. The smaller, more numerous structures that compose the gas-exchange region of the architecturally mature lung are designated alveoli (10). At autopsy, lung histology of infants with BPD is predominantly characterized by arrested lung development, including impaired alveolar and vascular growth (11-13). Similar structural abnormalities have been demonstrated in animal models of BPD (14,15). Thus, decrease alveolariztion and abnormal vascular growth are the central hallmarks of the “new BPD”, but mechanisms that inhibit lung growth in premature infants with severe BPD remain poorly understood. Therefore, to understand the mechanism of alveolarization can help us to realize the pathogenesis of BPD.

Alveolar capillary formation in the developing lung

During the period of alveolarization, the lung also undergoes marked vascular growth as reflected by the 20-fold increase in alveolar and capillary surface areas from birth to adulthood (16). Mechanisms that increase vascular surface area during late gestation and the early postnatal period are poorly understood, but it is clear that coordination of distal air space and vascular growth is essential for normal lung development. Previous studies have shown that many stimuli such as hypoxia, hyperoxia, or glucocorticoid treatment during the critical period of postnatal lung growth in the rat can disrupt alveolarization and cause lung hypoplasia. These models of lung hypoplasia are also associated with abnormalities of the pulmonary circulation (16-23). Recently, Jakkula M, et al. demonstrated that angiogenesis is necessary for normal alveolarization during a critical period of lung development in the rat (24).

Regulation of angiogenesis in developing lung

Blood vessels are constructed by two process: vasculogenesis, whereby a primitive vascular network is established during embryogenesis from multipotential mesenchymal proginators, and angiogenesis, in which preexisting vessels send out capillary sprouts to produce new vessels (22-25). Vascular endothelial growth factor (VEGF), the first characterized vascular specific growth factor, has been positioned as the most critical driver of vascular formation (26). Flk-1/KDR (VEGFR-2) and Flt-1 (VEGFR-1) are the endothelial specific tyrosine kinase receptors of VEGF (24,25). Bhatt AJ, et al. had demonstrated that disrupted pulmonary vasculature and decreased VEGF and Flt-1, but no changes in Flk-1, in human infants dying with BPD (26). Placenta growth factor (PlGF) belongs to the family of VEGF. It binds to the flt-1 VEGF receptor but not to the KDR/flk-1 receptor, which is thought to mediate most of the angiogenic and proliferative effects of VEGF. The PlGF mRNA was presented in most normal tissue, especially in placenta, thyroid, and lung (27). Recently, Carmeliet P, et al had demonstrated the synergism between VEGF and PlGF in angiogenesis and plasma extravasation in pathological conditions (28). However, the role of PlGF, another ligand of Flt-1, in lung development is unknown.

The role of PlGF in lung development

Recently, we found that overexpression of PlGF, using PGK promotor, seems disrupt pulmonary alveolarization. This partially mimics the pathological finding of BPD. However, this is not a good animal model of BPD because PlGF were overexpressed at all tissues. To solve this problem, we will use lung-specific promotor, SP-C (a gift from Dr. Whitsett at the University of Cincinnati), to produce targeted expression of PlGF transgenic mice. Therefore, we will understand the directed role of PlGF in lung development. In addition, we can use this model to study the mechanism of alveolarization and try to find a way to rescue the poor alveolarization of BPD. In the previous project (NSC91-2314-B002-187), we have finished the SP-C-PlGF construct and defined its function in vitro. The lung-specific PlGF overexpression transgenic mice will be generated in this year (2003).

In addition, RT-PCR revealed expression of *VEGF, Flt-1*, and *PlGF* in murine pulmonary type II epithelial cells (MLE-15) (data not showed). These data resemble that PlGF may play an autocrine or paracrine regulatory role in pneumocytes growth or differentiation during lung development. Further study should be performed to declare this hypothesis.

(二)研究目的：

第一年： Genotping for SP-C-PlGF transgenic mice and the role of PlGF on type II pneumocytes in vitro

(三)研究方法、進行步驟及執行進度。

1. 研究方法:

(1). Production of Transgenic Mice

Superovulated 3-4 week old FVB/N females will be produced by i.p. injections of 5 units pregnant mare serum followed 46 hours later by 5 units of human chorion gonadotropin. Females will then be mated with FVB/N males. At the time of detection of vaginal plug (assumed to be 0.5 days), the pregnant females will be sacrificed and the zygotes flushed put from the oviduct. After injection of the DNA into male pronucleus, the injected zygotes will be transferred to the oviducts of pseudopregnant females. DNA samples from the offspring will be isolated from about 50 mg of the tail to test for transgenics.

(2). Identifying Transgenics

The 10-30% of mice born which are identified as transgenic mice by removing about 50 mg from the tip of the tail and purifying DNA by modification of the procedure described by Hannahan (29). In order to disrupt tissue, digest protein and liberate the DNA, the tail tip is incubated overnight in an EDTA, SDS, and proteinase K solution at 62ºC. We then extract the solution with phenol, phenol-chloroform and then chloroform. The DNA is then spooled out with a glass rod, rinsed with ethanol, air-dried and dissolved in 200 ul H2O. This DNA now can be digested with most restriction enzymes. We typically digest with an enzyme that cuts at least once per unit in the molecular injected, to insure that possible large concatemers will be cut into predictable DNA segment sizes. The DNAs are electrophoresed, alkaline Southern blotted and hybridized to a random-primer radiolabeled probe. Transgenic animals are then detected by autoradiography.

(3) RT-PCR:

Total RNA is isolated using Trozol as per manufacturer's instructions from lung homogenates. RNA (0.5 ug per sample) is converted to complementary DNA (cDNA) by the oligo dT-primed reverse transcriptase (RT) reaction according to the manufacturer's protocol. Reverse-transcribed cDNA products is amplified by the polymerase chain reaction (PCR) with primers specific for PlGF or β-actin; for PlGF: 5'-TGG-ATG-AAT-ACC-CTG-ATG-AGG-TGT (sense primer) and 5'-CGA-GAG-AGA-AAG-AAA-GAA-GCC-AG (antisense primer); for β-actin: 5'-ATG-TAC-GTA-GCC-ATC-CAG-GC (sense primer) and 5'-AAG-GAA-GGC-TGG-AAA-AGA-GC.

PCR reactions consist of 10 pmol of each primer, 0.25 mM of each deoxynucleotide triphosphate, 2.5 mM MgCl2, 60 mM Tris-HCl (pH 8.5), 12.5mM $(NH4)_2SO_4$, and 0.1U Taq polymerase in a total reaction volume of 25 ul. PCR is performed in an thermocycler under the following conditions: 94℃ for 2 min, then 30 cycles of 94℃ for 30 s, 58℃ for 1 min and 72℃ for 1 min. The amplification products are separated by agarose gel electrophoresis and visualized by staining with ethidium bromide.

(4). Effects of PIGF on type II pneumocytes in vitro.

Mouse lung epithelial cell line (MLE-15) was a gift from Dr. Jeff Whitsett (University of Cincinnati).(30) The MLE-15 cell line maintains many of the morphological characteristics and gene expression patterns consistent with that seen in type II pneumocytes.(30,31) Cells are maintained in DMEM medium with 10% FBS. MLE-15 cells were seeded at 10^5/well in a 6-well plate and incubated at 37^0C in a 5% CO_2 atmosphere overnight. Different concentrations of rmPlGF or rmVEGF (up to 100 ng/ml) were added. Apoptosis of cells was measured by use of PI staining as described previously. Briefly, the cells were harvested 24 hours after cytokine treatment and washed with FACScan buffer (0.1% FBS in PBS) by centrifugation at 1,500 rpm for 10 min. The cells were then resuspended and fixed in 1.5 ml 70% ethanol solution at 4°C for a minimum of 30 min and washed twice with FACScan buffer. Then, 50 μg/ml PI was added and incubated at room temperature for 20 min. Cells were washed and resuspended in 200 μl FACScan buffer and analyzed on a FACSCalibur flow cytometer (BD Biosciences, California, USA). The percentage of apoptotic cells was determined by positive PI staining.

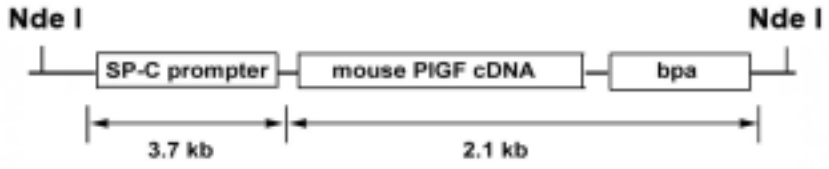
For cell proliferation assay, an enzyme immunoassay by quantification of BrdU incorporation into DNA was performed by use of cell proliferation ELISA, BrdU (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufacturer's instruction. Briefly, 10^4 cells were cultured in a 96-well microplate at 37℃ for 8 hours and then treated with or without various cytokines with serum starvated for 1 to 3 days. Subsequently, BrdU was added to the cells, and the cells were reincubated for 4 hours. During this labeling period, the pyrimidine analogue BrdU was

incorporated in place of thymidine into the DNA of proliferating cells. After removal of the culture medium, the cells were fixed and the DNA is denatured in one step by addition of FixDenat. The anti-BrdU-POD binds to the BrdU incorporated in newly synthesized cellular DNA. The immune complexes were detected by the subsequent substrate reaction. The reaction product was quantified by measurement of the absorbance at wavelengths of 370 nm-492 nm on a scanning multiwell spectrophotometer (ELISA reader). The developed color and thereby the absorbance values are directly correlated with the amount of DNA synthesis and thereby with the number of proliferating cells in the respective microcultures.

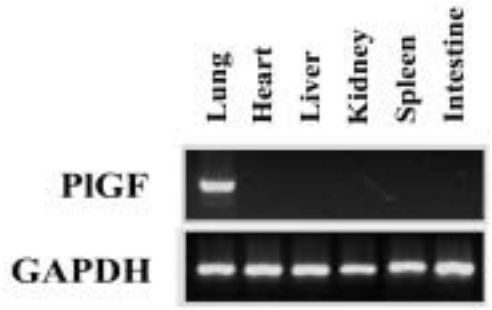
(四) 結果與討論:

1. **We have successfully generated and kept four different SP-C-PlGF transgenic lines with different mRNA expression levels, confirmed by real time RT-PCR.**

A.



B.



C.

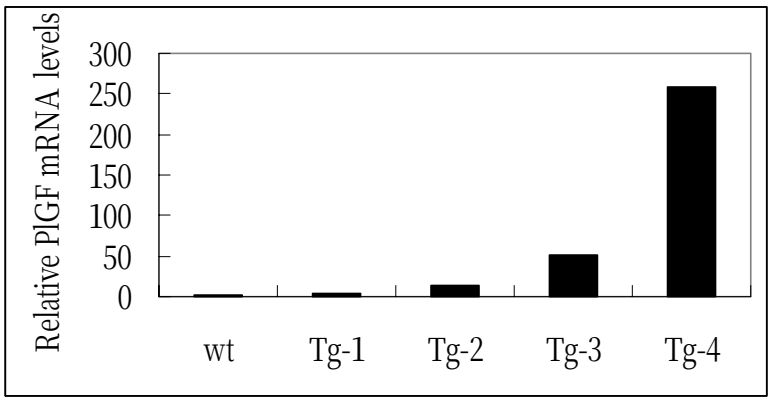


Figure 1. A. The map of SP-C promoter-mouse PlGF cDNA. B. Lung specificity of transgene expression was confirmed by PCR. C. Four different PlGF expression levels transgenic lines were confirmed by real time RT-PCR as compared with wild type mouse (Tg-1: 3.8-fold; Tg-2: 14-fold; Tg-3: 52-fold; Tg-4: 258-fold PlGF expression levels of wild type)

2. Exogenous PIGF promoted type II pneumocytes death and inhibited its proliferation in vitro.

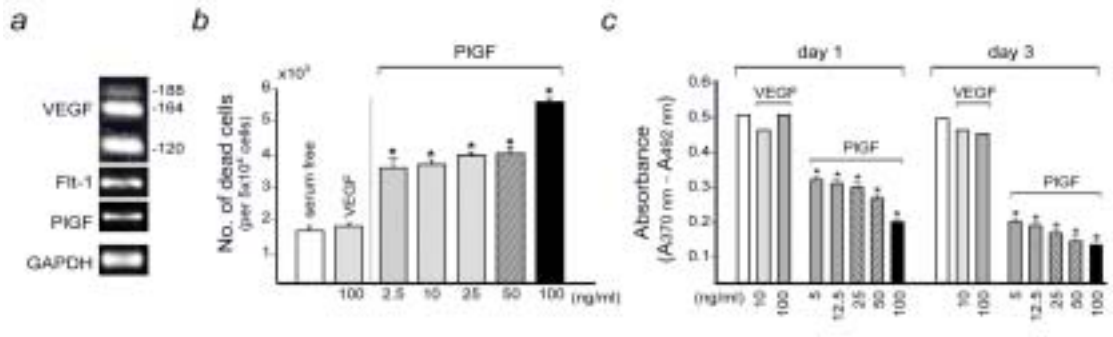


Figure 2. ***a,*** RT-PCR revealed expression of VEGF, Flt-1 and PlGF in murine pulmonary type II epithelial cell line (MLE-15). ***b,*** recombinant PlGF, but not recombinant VEGF, significantly enhanced cell death of MLE-15 cells. ***c,*** PlGF inhibited proliferation of MLE-15 cells in a dose-dependent fashion. Note that VEGF had no effects on MEL-15 cells.

3. Now, we will analyze the time course phenotype of this transgenic mouse, and no phenotype was noted when these transgenic mice were 2 months old.

(五)參考文獻

1. Northway WH Jr, rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. N Eng J Med 1967;276:357-68.
2. Truog WE, Prueitt JL, Woodrum DE. Unchanged incidence of bronchopulmonary dysplasia in survivors of hyaline membrane disease. J Pediatr 1978;92:261-4.
3. Horbar J, Soll RF, Sutherland JM, et al. A multicenter randomized, placebo-controlled trial of surfactant therapy for respiratory distress syndrome. N Eng J Med 1989;320:959-65.
4. Hack M, Horbar JD, Malloy MH, et al. Very low birth weight outcome of National Institute off Child Health and Human Development Neonatal Network. Pediatrics 1991;87:587-97.
5. Tsao PN, Wu TZ, Teng RJ, Tang JR, Yau TKI. 1998 Comparison of the outcome of extremely-low-birth-weight infants between two periods. Acta Paed Sin 39:233-6.
6. Bonikos DS, Bensch KG, Northway WH Jr. Oxygen toxicity in the newborn: the effect of chronic continuous 100 percent oxygen on the lungs of newborn mice. Am J Pathol 1976;85:623-35.
7. Deneke SM, Fanburg BL. Normobaric oxygen toxicity of the lung. N Eng J Med 1980;303:76-86.
8. Pappas CTE, Obara H, Bensch KG, Northway WH Jr. Effect of prolonged exposure to 80% oxygen on the lung of the newborn mouse. Lab Invest 1983;48:735-48.

9. Randell SH, Mercer RR, Young SL. Neonatal hyperoxia alters the pulmonary alveolar and capillary structure of 40-day-old rats. Am J Pathol 1990;136:1259-66.
10. Burri PH. The postnatal growth of the rat lung. III. Morphology. Anat Rec 1974;180:77-98.
11. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001;163:1723-1729.
12. Bland RD, Colason JJ (editors). Chronic lung disease of infancy. New York: Malcel Dekker;2000.
13. Hussain AN, Siddiqui NH, Stocker JT. Pathology of arrested acinar development in postsurfactant BPD. Hum Pathol 1998;29:710-717.
14. Coalson JJ, Winter VT, Siler-Khodr T, Yoder BA. Neonatal chronic lung disease in extremely premature baboons. Am J Respir Crit Care Med 1999;160:1333-1346.
15. Albertine KH, Jones GP, Starcher BC, Bohnsak JF, Davis PL, Cho S, Carton DP, Bland RD. Chronic lung injury in preterm lambs. Am J Respir Crit Care Med 1999;159:945-958.
16. Zelter TB, Caaduff JH, Gehr P, Pfenninger J, and Burri PH. The postnatal development and growth of the human lung. I. Morphometry. Respir Physiol 1987;67:247-267.
17. Le Cras TD, Kim DH, Gebb S, Markham NE, Shannon JM, Tuder RM, and Abman SH. Abnormal lung growth and the development of pulmonary hypertension in the Fawn-Hooded rat. Am J Physiol Lung Cell Mol Physiol 1999;277:L709-718.
18. Le Cras TD, Markham NE, Morris KG, Ahrens CR, McMurtry IF, and Abman SH. Neonatal dexamethasone treatment increases the risk for pulmonary hypertension in adult rat. Am J Physiol Lung Cell Mol Physiol 2000;278:L822-829.
19. Randell Sh, Mercer RR, and Ypung SL. Neonatal hyperoxia alters the pulmonary alveolar and capillary structure of 40-day-old rats. Am J pathol 1990;136:1259-1266.
20. Tang JR, Le Cras TD, Morris KG, and Abman SH. Brief perinatal hypoxia increases the severity of pulmonary hypertension after reexposure to hypoxia in infant rats. Am J Physiol Lung Cell Mol Physioil 2000;278:L356-364.
21. Jakkula M, Le Cras TD, Geeb S, Hirth KP, Tuder RM, Voelkel NF, and Abman SH. Inhibition of angiogenesis decreases alveolarization in the developing rat lung.
22. Risau W, Flamme I. Vasculogenesis. Annu Rev Cell Dev Biol 1995;11:73-91.
23. Folkman J, D'Amore PA. Blood vessel formation: what is its molecular basis? Cell 1996;87:1153-5.
24. Risau W. Mechanisms of angiogenesis. Nature 1997;386:671-4.
25. Hanahan D. Signaling vascular morphogenesis and maintenanced. Science 1997;277:48-50.
26. Bhatt AJ, Amin SB, Chess PR, Watkins RH, Maniscalco WM. Expression of Vascular endothelial growth factor and Flk-1 in developing and glucocorticoid-treated mouse lung. Pediatr Res 2000;47:606-613.

27. DiPalma T, Tucci M, Russo G, Maglione D, Lago CT, Romano A, Saccone S, Della Valle G, De Gregorio L, Dragani TA, Viglietto G, Persico MG. The placenta growth factor gene of the mouse Mam Genome 1996;7(1):6-12
28. Carmeliet P, Moons L, Luttun A, et al. Synergism between vascular endothelial growth factor and placenta growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nature Med 2001;7(5):575-583.
29. Hannahan D. Heritable formation of pancreatic beta-cell tumors in transgenic mice expressing recombinant insulin/simian virus 40 oncogene. Nature 1985;315:115-122.
30. Wikenheiser, K.A., et al., Production of immortalized distal respiratory epithelial cell lines from surfactant protein C/simian virus 40 large tumor antigen transgenic mice. Proc Natl Acad Sci U S A, 1993. 90(23): p. 11029-33.

計畫成果自評

1. 目前計畫進展順利，我們已按照計畫進度於第一年成功建立肺臟獨特過度表現胎盤生長因子的基因轉殖鼠，並已往下繁殖四個不同基因表現量的基因轉殖鼠。另外，我們亦已證實外加胎盤生長因子蛋白會促進肺臟上皮細胞死亡及抑制其增生，顯見過量的胎盤生長因子對肺臟應有不好的影響。

2. 若接下來能建立此四個不同基因表現量的基因轉殖鼠(homozygous)，我們便可以看在不同年齡的小鼠肺臟的發育或構造是否受影響，同時此肺臟結構的改變程度是否和胎盤生長因子的表現量相關，這樣更可相信若在病理情況下引起胎盤生長因子的過度表現和肺臟結構的修復或傷害是有其因果關係的。這樣應可當作慢性肺疾病的動物模式來發表論文。

3. 另外，我們也可從四個不同基因表現量的基因轉殖鼠中，挑選出發病較早且較嚴重的基因轉殖鼠作為我們的動物模式來研究肺泡的修復及再生的機轉，藉以提供日後治療此疾病的方式。

4. 再者，我們也想找到一發病較晚甚至不發病的肺臟獨特過度表現胎盤生長因子的基因轉殖鼠，如此可提供我們研究胎盤生長因子和肺臟相關疾病的探討，並可以瞭解胎盤生長因子在這些肺臟相關疾病的病裡成因中是扮演什麼樣的角色。