行政院國家科學委員會補助
大專學生參與專題研究計畫研究成果報告

計畫名稱：Association between FAS polymorphism and lung cancer risk in Taiwan

執行計畫學生：孫盟勝
學生計畫編號：NSC 98-2815-C-040-019-B
研究期間：98 年 07 月 01 日至 99 年 02 月 28 日止，計 8 個月
指導教授：李輝

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

執行單位：中山醫學大學醫學系

中華民國 99 年 03 月 31 日

行政院國家科學委員會補助

大專學生參與專題研究計畫研究成果報告

＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊
＊ 計畫 ＊
＊ ：**Association between FAS polymorphism and lung cancer risk in Taiwan** ＊
＊ 名稱 ＊
＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊＊

執行計畫學生：**孫盟勝**

學生計畫編號：NSC　98-2815-C-040-019-B

研 究 期 間 ：**98 年 7 月 1 日至　99 年 2 月底止，計 8 個月**

指 導 教 授 ：**李　輝教授**

處理方式(請勾選)：□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：中山醫學大學醫學分子毒理學研究所

中華民國　　99 年　　03 月　　28 日

(一)摘要

Fas/FasL is a well known pathway to regulate cell death and it is the receptor and ligand of the tumor necrosis factor family. Apoptotic gene alteration may increase cancer risk mediated through the cytolytic activity of killer cells and/or individual susceptibility to environmental carcinogens. Previous studies indicated that Fas polymorphism play a role in the prognostic value in various human cancers including lung carcinomas. The association between Fas polymorphism and cancer risk and clinical outcome has been extensively studied. In this study, we will ask whether the polymorphism of Fas gene could contribute to lung cancer risk and patients' clinical outcome in Taiwanese. Results showed Fas -670 AA genotype carriers had a 1.67 fold excess risk of NSCLC compared with Fas -670GG. This result demonstrates that Fas -670A/G polymorphism is associated with NSCLC risk.

(二)研究動機與研究問題

Lung cancer is the leading cause of cancer death in the world. Moreover, the incidence rate of lung cancer is high and the overall 5-year survival is less than 16% (Sugimura et al., 2006). Apoptotic cell death is the common mechanism to kill the unwanted cells including cancer. Fas receptor (Fas, CD95, APO-1) and Fas ligand (FasL, CD95L) are well known in cell death pathway belong to the tumor necrosis factor family of receptors and ligands, respectively. Functional single nucleotide polymorphisms (SNP) of Fas -670A/G is located in the enhancer region (Huang et al., 1997). Studies have shown that SNP of Fas -670A/G is associated with cancer risk in lung cancer (Park et al., 2006; Zhang et al., 2005). In addition, their prognostic significance in esophageal cancer (Jain et al., 2007), T cell leukemia (Farre et al., 2007), and cervical cancer (Lerma et al., 2008) are estimated previously. However, no report demonstrates the role of Fas -670A/G in non-small-cell lung cancer (NSCLC) in Taiwan. In this study, we evaluated whether Fas -670A/G polymorphisms were associated with NSCLC.

(三)研究方法及步驟

Patients and Healthy Individuals

This case-control study consisted of 308 lung cancer patients and 308 cancer-free controls. All subjects were unrelated ethnic Chinese and residents in central Taiwan. Lung cancer patients were diagnosed as adenocarcinoma (162; 52.6%) or squamous cell carcinoma (146; 47.4%) at division of thoracic surgery, Taichung Veterans General Hospital between 1993 and 2004. All patients and controls were informed and ask for written consent approved by

the Institutional Review Board.

Genomic DNA Extraction

Genomic DNA was extracted from peripheral blood cells of all healthy control subjects by conventional methods. Samples of patients from surgically resected normal tissue adjacent to the lung tumor were prepared by using proteinase K digestion and phenol-chloroform extraction followed by ethanol precipitation.

PCR-RFLP Analysis for Fas -670A/G Genetic Polymorphism

Genotypes of Fas -670A/G was distinguished by PCR-RFLP as described by Lee et al (Lee et al. 2001). Samples were loaded onto 3 percent agarose gel, stained with ethidium bromide, and visualized under ultraviolet illumination. After digestion, Fas -670A/G genotype was determined with A allele by 232 bp (232bp and 99 bp) and G allele by 188 bp (188 pb, 99 bp and 44 pb) fragment length patterns.

(四)結果與討論

Distribution of overall case-control characteristics are summarized in Table 1. There was no significantly difference between patients and controls in gender and smoking habit distribution. The average age of both case and control group were 64.3 years. Among these lung cancer patients, 162 (52.6%) patients were diagnosed as adenocarcinoma and 146 (47.4%) were squamous cell carcinoma.

The genotypic and allelic frequencies of Fas -670A/G polymorphism and its association with the risk of NSCLC are described in Table 2. Genotypic frequencies of Fas -670 AA was significantly higher in patients than that in controls ($p = 0.03$). Multiple logistic regression analysis showed that the Fas -670AA genotype carriers had a 1.67 and 1.62 fold excess risk of lung cancer development compared with Fas -670GG and Fas -670GG/AA carriers, respectively (adjusted OR 1.67, 95% CI 1.04 to 2.69; adjusted OR 1.62, 95% CI 1.16 to 2.27). Moreover, higher NSCLC risk in males (adjusted OR 1.58, 95% CI 1.06 to 2.36), nonsmokers (adjusted OR 1.80, 95% CI 1.15 to 2.82), and patients with adenocarcinoma (adjusted OR 1.70, 95% CI 1.14 to 2.54) were found. These results indicate that Fas -670AA carriers might associate with high NSCLC risk especially in males, nonsmokers and adenocarcinomas.

It is well known that Fas is expressed in many tissues, but FasL is naturally produced by activated cytotoxic T cells and natural killer cells. The interaction of Fas and FasL recruits

Fas associated death domain (FADD) and results in the molecular mechanism of activation induced cell death (Depraetere et al., 1997; Ju et al., 1995). Cancer development needs kinds of mechanisms to get tumor growth forward and keep immunosurveillance (Hanahan et al., 2000). Many cancer cells are found to express FasL and may contribute to the immune privilege of tumors, including lung cancer (Niehans et al., 1997; Viard-Leveugle et al., 2003). In addition, it has been reported that only apoptosis-resistant cancer cells express FasL (Igney et al., 2002). In this case, T cells with sensitivity to Fas-mediated cell death may be eliminated (Restifo et al., 2001). Consequently, FasL, derived from tumors, extends immune evasion from tumor site to peripheral sites and results in malignant progression and metastasis (Kim et al., 2006; Kim et al., 2007). In our result, higher NSCLC risk of Fas -670AA carriers might due to higher Fas expression on T cell which resulting in T cell apoptosis. However, we need cellular evidences to support our suggestion.

(五)參考文獻

Depraetere V, Golstein P. Fas and other cell death signaling pathways. Semin Immunol. 1997 Apr;9(2):93-107. Review.

Farre L, Bittencourt AL, Silva-Santos G, Almeida A, Silva AC, Decanine D, Soares GM, Alcantara LC Jr, Van Dooren S, Galvão-Castro B, Vandamme AM, Van Weyenbergh J. Fas 670 promoter polymorphism is associated to susceptibility, clinical presentation, and survival in adult T cell leukemia. J Leukoc Biol. 2008 Jan;83(1):220-2. Epub 2007 Oct 25.

Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA. Fas ligand-induced apoptosis as a mechanism of immune privilege. Science. 1995 Nov 17;270(5239):1189-92.

Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000 Jan 7;100(1):57-70. Review.

Huang QR, Morris D, Manolios N. Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene. Mol Immunol. 1997 Jun;34(8-9):577-82.

Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer. 2002 Apr;2(4):277-88. Review.

Jain M, Kumar S, Upadhyay R, Lal P, Tiwari A, Ghoshal UC, Mittal B. Influence of apoptosis (BCL2, FAS), cell cycle (CCND1) and growth factor (EGF, EGFR) genetic polymorphisms on survival outcome: an exploratory study in squamous cell esophageal

cancer. Cancer Biol Ther. 2007 Oct;6(10):1553-8. Epub 2007 Jul 7.

Ju ST, Panka DJ, Cui H, Ettinger R, el-Khatib M, Sherr DH, Stanger BZ, Marshak-Rothstein A. Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation. Nature. 1995 Feb 2;373(6513):444-8.

Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. Immunology. 2007 May;121(1):1-14. Epub 2007 Mar 26. Review.

Kim R, Emi M, Tanabe K, Arihiro K. Tumor-driven evolution of immunosuppressive networks during malignant progression. Cancer Res. 2006 Jun 1;66(11):5527-36. Review.

Lee YH, Kim YR, Ji JD, Sohn J, Song GG. Fas promoter -670 polymorphism is associated with development of anti-RNP antibodies in systemic lupus erythematosus. J Rheumatol. 2001 Sep;28(9):2008-11.

Lerma E, Romero M, Gallardo A, Pons C, Muñoz J, Fuentes J, Lloveras B, Catasus L, Prat J. Prognostic significance of the Fas-receptor/Fas-ligand system in cervical squamous cell carcinoma. Virchows Arch. 2008 Jan;452(1):65-74. Epub 2007 Nov 14.

Niehans GA, Brunner T, Frizelle SP, Liston JC, Salerno CT, Knapp DJ, Green DR, Kratzke RA. Human lung carcinomas express Fas ligand. Cancer Res. 1997 Mar 15;57(6):1007-12.

Park SH, Choi JE, Kim EJ, Jang JS, Lee WK, Cha SI, Kim CH, Kam S, Kim DS, Park RW, Kim YC, Han SB, Jung TH, Park JY. Polymorphisms in the FAS and FASL genes and risk of lung cancer in a Korean population. Lung Cancer. 2006 Dec;54(3):303-8. Epub 2006 Oct 2.

Restifo NP. Countering the 'counterattack' hypothesis. Nat Med. 2001 Mar;7(3):259.

Sugimura H, Yang P. Long-term survivorship in lung cancer: a review. Chest. 2006 Apr;129(4):1088-97. Review.

Sun T, Miao X, Zhang X, Tan W, Xiong P, Lin D. Polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma. J Natl Cancer Inst. 2004 Jul 7;96(13):1030-6.

Viard-Leveugle I, Veyrenc S, French LE, Brambilla C, Brambilla E. Frequent loss of Fas expression and function in human lung tumours with overexpression of FasL in small cell lung carcinoma. J Pathol. 2003 Oct;201(2):268-77.

Wu J, Metz C, Xu X, Abe R, Gibson AW, Edberg JC, Cooke J, Xie F, Cooper GS, Kimberly RP. A novel polymorphic CAAT/enhancer-binding protein beta element in the FasL gene promoter alters Fas ligand expression: a candidate background gene in African American systemic lupus erythematosus patients. J Immunol. 2003 Jan 1;170(1):132-8.

Zhang X, Miao X, Sun T, Tan W, Qu S, Xiong P, Zhou Y, Lin D. Functional polymorphisms in cell death pathway genes FAS and FASL contribute to risk of lung cancer. J Med Genet. 2005 Jun;42(6):479-84

(六)表格

Table 1. Distributions of select characteristics by case-control status.

Parameter	Case n(%)	Control n(%)	P value[1]	OR value[2] (95%CI)
Total	308	308		
Age (yr ±SD)	64.3±10.3	64.3±10.7	0.68	1.00(0.99-1.02)
Gender				
Male	213(69.2)	213(69.2)	0.99	1.00(0.71-1.40)
Female	95(30.8)	95(30.8)		
Smoking status				
Smoker	129(41.9)	129(41.9)	0.99	1.00(0.73-1.38)
Nonsmoker	179(58.1)	179(58.1)		
Histological type				
adenocarcinoma	162(52.6)			
squamous cell carcinoma	146(47.4)			

[1]Two sided Chi-square test, [2] Multiple logistic regression analysis.

Table 2. Genotypic frequencies of FAS -670A/G polymorphism among patients and controls and their associations with the risk of lung cancer

FAS polymorphism	Case n(%)	Control n(%)	P value [1]	OR[2] (95%CI)
All subject	308	308		
G/G	47(15.3)	57(18.5)	0.02	1.00
A/G	141(45.8)	164(53.2)		1.04(0.67-1.63)
A/A	120(39.0)	87(28.2)		1.67(1.04-2.69)
G/G+G/A	188(61.0)	221(71.8)	0.01	1.00
A/A	120(39.0)	87(28.2)		1.62(1.16-2.27)
Female	95	95		
G/G	13(13.7)	11(11.6)	0.15	1.00
A/G	47(49.5)	60(63.2)		1.61(0.57-4.53)
A/A	35(36.8)	24(25.3)		1.69(0.60-4.77)
G/G+G/A	60(63.2)	71(74.7)	0.09	1.00
A/A	35(36.8)	24(25.3)		1.73(0.93-3.22)
Male	213	213		
G/G	34(16.0)	46(21.6)	0.06	1.00
A/G	94(44.1)	104(48.8)		1.15(0.54-2.44)
A/A	85(39.9)	63(29.6)		1.00(0.47-2.14)
G/G+G/A	128(60.1)	150(70.4)	0.03	1.00
A/A	85(39.9)	63(29.6)		1.58(1.06-2.36)
Smoking	129	129		
G/G	22(17.1)	30(23.3)	0.31	1.00
A/G	57(45.7)	59(44.2)		1.32(0.68-2.55)
A/A	50(38.8)	40(31.0)		1.71(0.86-3.40)
G/G+G/A	79(61.2)	89(69.0)	0.19	1.00
A/A	50(38.8)	40(31.0)		1.41(0.84-2.36)
Nonsmoking	179	179		
G/G	25(14.0)	27(15.1)	0.03	1.00
A/G	84(46.9)	105(58.7)		0.86(0.47-1.60)
A/A	70(39.1)	47(26.3)		1.61(0.83-3.11)
G/G+G/A	109(60.9)	132(73.7)	0.01	1.00
A/A	70(39.1)	47(26.3)		1.80(1.15-2.82)
Adenocarcinoma	162	308		
G/G	20(12.3)	57(18.5)	0.02	1.00
A/G	77(47.5)	164(53.2)		1.34(0.75-2.38)
A/A	65(40.1)	87(28.2)		2.13(1.17-3.89)
G/G+G/A	97(59.9)	221(71.8)	0.01	1.00
A/A	65(40.1)	87(28.2)		1.70(1.14-2.54)

Squamous cell carcinoma	146	308		
G/G	27(18.5)	57(18.5)	0.10	1.00
A/G	64(43.8)	164(53.2)		0.82(0.48-1.42)
A/A	55(37.7)	87(28.2)		1.34(0.76-2.36)
G/G+G/A	91(62.3)	221(71.8)	0.04	1.00
A/A	55(37.7)	87(28.2)		1.54(1.01-2.33)

[1]Two sided Chi-square test, [2] Multiple logistic regression analysis.