# TGF 乙型細胞激素對人類呼吸道上皮細胞產生扁平化變性增生之機轉：著重普羅林氨酸小分子蛋白之表現

計畫編號：NSC88-2314-B002-249
計畫主持人：廖永祥
執行期間：87.8.1.～88.7.31.
執行單位：台大醫學院內科

中文摘要

肺癌已成為臺灣女性癌症病人死亡原因第一位。而扁平異形增生是被認為與肺癌形成相關，而吡喀氨酸小分子量蛋白質(SPRR1)與扁平異行增生有關。本實驗為探討正常及癌轉形呼吸道細胞是否在 SPRR1 基因是否有不同的表現。藉著北方及西方墨點技術分析發現，我們表現 SPRR1 基因有不同的表現：人類原位呼吸道細胞在基礎狀態下，有很高的表現，然而轉形的 HBE 細胞，BEAS-2B S 細胞，A549 肺腺癌細胞其 SPRR1 基因表現甚低或全無。在肺癌細胞中，只有 H178 腺扁平癌細胞及 H174 乳突形腺癌細胞在 TPA 刺激下，全表現 SPRR1 基因。簡言之，SPRR1 基因表現在各種肺癌細胞異有個別差異性存在。

**Abstract**

Lung cancerhas become the leading cause of female cancer death in Taiwan. To further understand of differences in SPRR1 gene expression between normal and transformed airway epithelial cells, studies were undertaken to compare the expression of SPRR1 in different airway cells and lung cancer cell lines by using Northern blot and Western blot analysis. Our study showed different expression of SPRR1gene product in various lung airway epithelial cells by Northern blot and Western blot analysis; human primary TBE cells had high expression of SPRR1 gene product in basal status; whereas, transformed HBE, BEAS-2B S cells and A549 lung adenocarcinoma cells showed low or absence of SPRR1 gene expression. In lung cancer cells, TPA-induced SPRR1 expression was found only in H178 adenosquamous carcinoma cell and H174 papillary adenocarcinoma. In summary, the SPRR1 gene expression in lung cancer cells is individualized and heterogeneous.

**Background and Aims:**

Lung cancer, mostly caused by active smoking, has become the leading cause of female cancer death, and second in male, in Taiwan. It has been suggested tha squamous metaplasia and dysplasia play an important role in airway epithelia cell transformation and that preneoplastic cells have a clonal growth advantage over normal cells, and finally suing the carcinoma (Pfeifer AMA, 1989). The small proline-rich protein (SPR) family, induced by UV irradiation and TPA were first reported in human keratinocyte cultures (Kartasova, 1988). Recently SPRR1 has been reported to be an early marker associated with squamous cel differentiation of airway epithelium (Tesfaigzi, 1990; An 1992; Reddy, 1995; Tesfaigzi, 1996). Since loss of SPRR1 expression may be a marker of loss of cell differentiation, we hypothesized that loss of SPRR1 expression in airway epithelia

is a sign of lung cancer transformation. To further understand of differences in SPRR1 gene expression between normal and transformed airway epithelial cells, studies were undertaken to compare the expression of SPRR1 in normal tracheobronchial epithelial cells (TBE cells), transformed human bronchial epithelial cells (HBE cells), and lung cancer cell lines by using Northern blot analysis and Western blot analysis.

**Results and Discussions**

*Expression of SPRR1 gene product in different airway epithelial cell type*

Our study showed that human primary TBE cells had high expression of SPRR1 gene product in Northern blot analysis in basal status; whereas, transformed HBE, BEAS-2B S cells, and A549 lung adenocarcinoma cells showed low or absence of SPRR1 gene expression. In Western blot analysis, staining with polyclonal antibody to SPRR1 protein was strongly seen only in primary human TBE cells. The expression of SPRR1 protein in transformed HBE was modest, and A549 lung adenocarcinoma, absent.

*TPA Response in Different Airway Epithelial Cells*

In Northern blot analysis, primary human TBE cells, and transformed HBE cells showed increased SPRR1 expression after TPA treatment. In lung cancer cells, TPA-induced SPRR1 expression was found only in H178 adenosquamous carcinoma cell and H174 papillary adenocarcinoma; whereas there were no expression of SPPR1 gene product in basal status and after TPA stimulation of other lung cancer cell lines, including A549 adenocarcinoma cells, H182 squamous cell carcinoma cells, 5935 lung carcinoma, H358 bronchoalveolar cell carcinoma cells. In Western blot analysis, human primary TBE cells and transformed HBE cells responded strongly to TPA stimulation. In lung cancer cells, only H174 papillary adenocarcinoma demonstrated a weak response to TPA treatment. Other lung cancer cells showed no respone to TPA stimulation.

In Northern and Western blot analysis, we found SPRR1 gene expression in human primary TBE cells and transformed HBE cells, whereas most of lung cancer cells lost the expression of SPRR1, except H174 papillary adenocarcinoma and H178 adenosquamous cell carcinoma. The expression of SPRR1 gene could be increased by TPA-treatment in human primary TBE cells, transformed HBE cells, H174 and H178 cells. Interestingly, TPA has no effect in most lung cancer cells, including A549, H182, H358, and 5935. The TPA effect for SPRR1 expression in cancer cells remain little known. Owen et al reported that TPA produced a large increase of SPRR1 protein and mRNA in papilloma and squamous cell carcinoma (Owen DM, Zainal TA, 1996). However, our study showed that the response of TPA for SPRR1 expression in lung cancer cells is heterogeneous. In a tumorigenicity study, using malignant keratinocyte from head and neck squamous cell carcinoma, restoration of wild type p53 (wt-p53) suppressed the tumor growth in nude mice. Interestingly, exogeneous wt-p53 increased baseline mRNA expression of SPRR1, a characteristic of prodifferentiating effect (Courtois S, 1997). This study implicated loss of SPRR1 expression in premalignant lesion could be a marker of carcinogenesis in progress. On the contrary, our study showed most lung cancers, including A549, H182, H358, 5935, lose of SPRR1

expression, except H174 and H178. As our study, the expression of esophagin, homologue to SPRR1, was reported to restricted to normal esophageal mucosa, not detectable in esophageal squamous cell carcinoma. (Abraham JM, Wang S, 1996).

**Assessment of Our Results**

The SPRR1 gene expression in lung cancer cells is individualized and heterogeneous. By Northern blot analysis and Western blot analysis, we found that most lung cancer cells including A549 adenocarcinoma, H182 squamous cell carcinoma, H352 bronchioloaleveolar cell carcinoma, and H5935 adenocarcinoma lose the expression of SPRR1 gene expression. Only H174 papillary adenocarcinoma and H178 adenosquamous cell carcinoma still express the SPRR1 gene. We need further to understand the molecular mechanisms of regulation in gene transcription, by using footprinting assay and gel mobility shift assay.

**Reference**


Abraham JM, Wang S, Suzuki H, Jiang HY, Rosenblum-Vos LS, Yin J, Meltzer SJ. Esophagin cDNA cloning and characterization: a tissue-specific member of the small proline-rich protein family that is not expressed in esophageal tumors. Cell Growth Differ 1996;7:855-860.

An G, Tesfaigzi J, Carlson DM, Wu R. Expression of a squamous cell marker, the spr1 gene, is posttranscriptionally down-regulated by retinol in airway epithelium. J Cell Physiol 1993;157:562-568

Chomczynski, P., and N. Sacchi. Single-step of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162;1987: 1546-159.

Courtois SJ, Woodworth CD, Degreef H, Garmyn M. Early ultraviolet B-induced G1 arrest and suppression of the malignant phenotype by wild-type p53 in human squamous cell carcinoma cells. Exp Cell Res 1997;233:135-144.

Ishida-Yamamoto A, Kartasova T, Matsuo S, Kuroki T, Iizuka H. Involucrin and SPRR are synthesized sequentially in differentiating cultured epidermal cells. J Invest Dermatol 1997;108:12-16.

Kartasova T, Ponec M, van de Putte P. Induction of proteins and mRNAs after uv irradiation of human epidermal keratinocytes. Exp Cell Res 1988;174:421-432.

Owens DM, Zainal TA, Jetten AM, Smart RC. Localization and expression of cornifin-alpha/SPRR1 in mouse epidermis, anagen hair follicles, and skin neoplasms. J Invest Dermatol 1996;106:647-654.

Pfeifer AM, Lechner JF, Masui T, Reddel RR, Mark GE, Harris CC. Control of growth and squamous differentiation in normal human bronchial epithelial cells by chemical and biological modifiers and transferred genes. Environ Health Perspect 1989;80:209-220.

Reddy SP, Chuu YJ, Lao PN, Donn J, Ann DK, Wu R. Expression of human squamous cell differentiation marker, SPR1, in tracheobronchial epithelium

depends on JUN and TRE motifs. J Biol Chem 1995;270:26451-26459.

Robinson CB, Wu R. Culture of conducting airway epithelial cells in serum-free medium. J Tiss Cult Meth 1991;13:95-102.

Tesfaigzi J, An G, Wu RE, Carlson DM. Induction in the expression of an unusual proline-rich protein by pig tracheal surface epithelial cells maintained in primary culture. Biochem Biophys Res Commun 1990;172:1304-1309.

Tesfaigzi J, Th'ng J, Hotchkiss JA, Harkema JR, Wright PS. A small proline-rich protein, SPRR1, is upregulated early during tobacco smoke-induced squamous metaplasia in rat nasal epithelia. Am J Respir Cell Mol Biol 1996;14:478-486.