行政院國家科學委員會專題研究計畫期中報告

肺癌轉移抑制基因–CRMP-1 的上下游調控基因之分析

The upstream and downstream genes of CRMP-1, an invasion suppressor gene

計畫編號：NSC91-2314-B-002-267

執行期限：91 年 8 月 1 日至 92 年 7 月 31 日

主持人: 施金元　　國立台灣大學醫學院內科

共同主持人：楊泮池　國立台灣大學醫學院內科

一、中文摘要

目前肺癌已是台灣以及世界各國最嚴重的癌症死亡原因。癌轉移是目前外科手術、放射治療、化學治療，各種治療方法失敗的主要原因。我們希望研究癌轉移機制，尋找與肺癌轉移有關的基因，來改善治療的方法。利用已建立之高侵襲及轉移傾向和低侵襲及轉移傾向之各類腺癌細胞株(CL1-0, CL1-1, CL1-5, and CL1-F4)、cDNA 微陳列技術，找尋與癌轉移相關之新基因。我們證實 collapsin response mediator protein-1 (CRMP-1) 是一癌轉移抑制基因。

之前學者已發現 cyclooxygenase-2 (COX-2)會促進癌轉移，促進癌細胞生長、抑制細胞凋亡，以及增加 actin polymerization，進而增進細胞的活動力及侵襲力，最終使癌細胞轉移。CRMP-1 與 COX-2 在許多方面，有著相反的功能。所以我們想了解 CRMP-1 與 COX-2 之間的關係。初步的研究發現 COX-2 表現會使 CRMP-1 表現量減少；而加入 COX-2 的抑制劑，可使 CRMP-1 表現增加。我們設計一系列實驗來釐清 CRMP-1 與 COX-2 的交互作用。

關鍵詞：肺腺癌、轉移機轉、CRMP-1, COX-2

Abstract

Lung cancer is the leading cause of cancer mortality in most countries, including Taiwan. Metastasis is the most feared manifestation hat defeats the present modalities of treatments, such as surgery, radiation and chemotherapy. We are interested in studying specific mechanisms involving the metastasis in lung cancer and searching for new genes associated with the invasion of lung cancer, in order to improve the treatment modality. We used a high-density cDNA microarray to identify and then confirm CRMP-1 is an invasion-suppressor genes. Accumulating evidence has suggested that cancer cells expressing higher levels of COX-2 would obtain higher invasive abilities. CRMP-1 and COX-2 seem to work in opposite way. This study plans to investigate whether COX-2 would alter the cellular expression of CRMP-1 in human lung adenocarcinoma cell lines or vice versa. Preliminary results show that COX-2 inhibitor induces the expression of CRMP-1 and expression COX-2 suppresses the expression of CRMP-1.

Keywords: Lung adenocarcinoma, metastasis, CRMP-1, COX-2

二、背景與目的

Lung cancer is a serious health problem in Taiwan. The death rate due to lung cancer has increased tremendously in both sexes in the past 30 years. The rate of increase in lung cancer mortality in Taiwan is highest in the world. Surgical removal is the hope to be successful in the treatment of primary lung cancer, but widespread dissemination often defeats this mode of treatment. Metastasis spread is common in lung cancer patients, especially small cell lung cancer and adenocarcinoma. Wide spread dissemination is common in liver, bone, bone marrow, adrenal gland and brain. Although improvements have been made concerning earlier detection, chemotherapy and radiotherapy, most of the lung cancer patients still die of distant metastases. The mechanism why lung cancer has higher potential for systemic metastasis is not known. We attempt to do some studies in metastasis research and further understand the biology of this process. An improved understanding of the pathogenesis of metastasis at the molecular level will lead to the design of new strategies of treatment: such as transfer of invasion-suppressing genes or anti-sense constructs, as well as interference with signal transduction pathways.

Tumor dissemination is a complex process. The pathogenesis of cancer metastasis consists of a series of linked, sequential, and selective steps. From a single parental tumor one can isolate sublines that exhibit different metastatic capacities. When these differences are relatively stable, comparisons can be made between the variants to determine factors responsible for these phenotypic differences. Several approaches have been taken to search for genes that involved in metastasis. To investigate the mechanisms involving the metastasis in lung adenocarcinoma, we have isolated several sublines from a lung adenocarcinoma cell lines by repeated selection of more invasive cells. These selected sublines have shown increased invasive as well as metastatic potential compared to the parental cells. We also use these cell lines to investigate the new genes controlling the metastasis by cDNA microarray system. One candidate gene--collapsin response mediator protein-1 (CRMP-1) decreased in high invasive cell lines CL1-5 and CL1-F4. In vitro invasion assay revealed decline of invasion ability after transfection of CRMP-1 cDNA into CL1-5. We collected clinical information of the lung cancer patients and tissues and confirmed that CRMP-1 might be an invasion-suppressing gene.

Specific Aims

Identify the upstream regulator and downstream effector of CRMP-1 in invasion-suppression.

三、方法與結果

I Expression of CRMP-1 and COX-2 mRNA in CL1-0, CL1-1, CL1-5, and CL1-5-F4 cells

Lung adenocarcinoma cell lines with different invasive activities (CL1-0 cells and its sublines CL1-1, CL1-5, and CL1-5-F4 cells, in ascending order of activity) was established in our laboratory. Real-time

quantitative RT-PCR was performed to quantitate the mRNA of CRMP-1 and COX-2 in each clone (CL1-0, CL1-1, CL1-5, and CL1-5-F4). The expression of CRMP-1 was inversely correlated with the invasiveness (Figure 1A) and the expression of COX-2 was positively correlated with the invasiveness of cell lines (Figure 1B).

Fig 1A

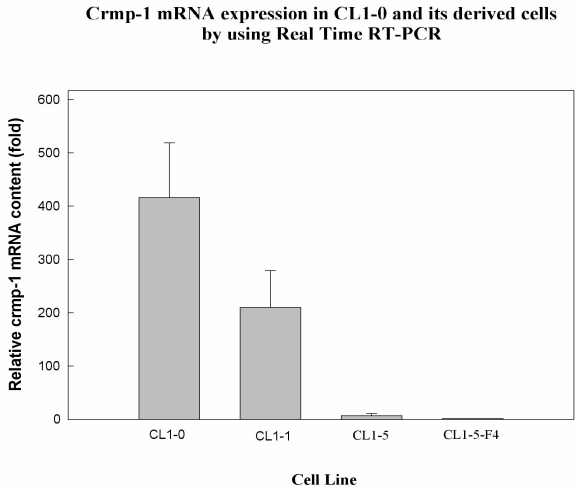
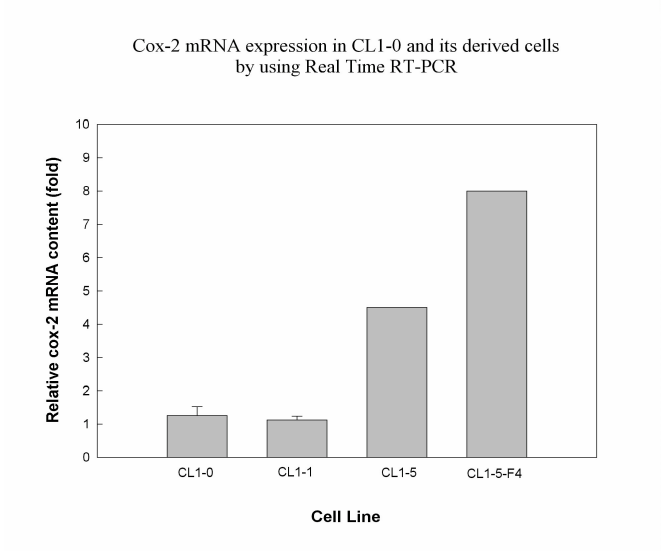


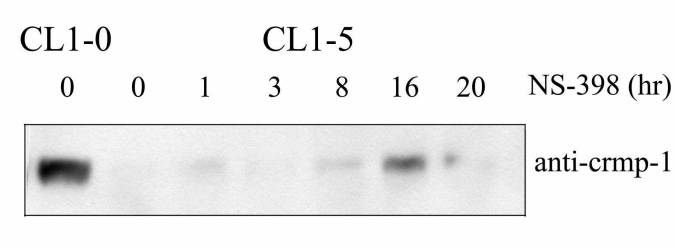
Fig 1B



II. Treatment of selective COX-2 inhibitor, NS-398, induces the expression of CRMP-1 in CL1-5

We treated CL1-5 with selective COX-2 inhibitor and evaluated the expression of CRMP-1 with our CRMP-1 specific monoclonal antibody-Y21. The expression of CRMP-1 protein was induced maximally 16 hours after the addition of NS-398 (Figure 2. These data suggested that inhibition of COX-2 activity could increase the amount of CRMP-1 protein in high invasive CL1-5 cells.

Fig 2



III. The expression of COX-2 and CRMP-1 mRNA in COX-2 transfected CL1-0 cells

Clone Stable 17 was a stable transfectant of COX-2 into CL1-0 cell. We used quantitative real-time RT-PCR to quantitate the mRNA amount of COX-2 and CRMP-1 in clone stable 17. The vector-only transfectant (mock vector transfected into CL1-0, label as neo) was used as a control. The Figure 3 shows that after transfection of COX-2 into CL1-0, the expression of COX-2 in clone stable 17 was up to the same level as that in CL1-5 (Figure 3A), and the expression of CRMP-1 was down regulated to about the same level as in CL1-5 (Figure 3B). Mock transfectant (Neo) did not have these changes. This suggested COX-2 suppressed the expression of

CRMP-1.

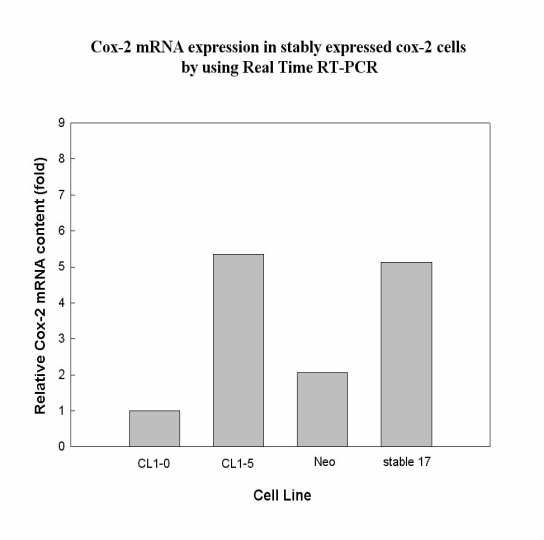


Fig 3A

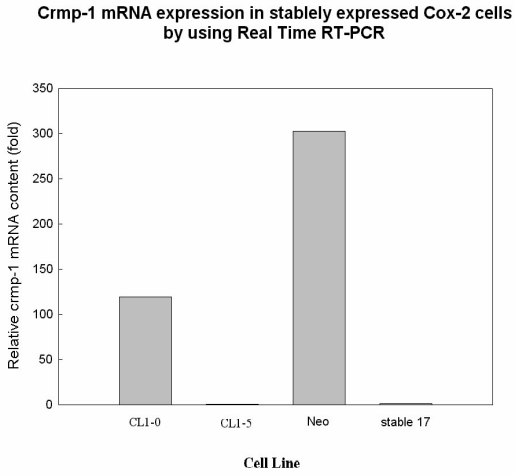
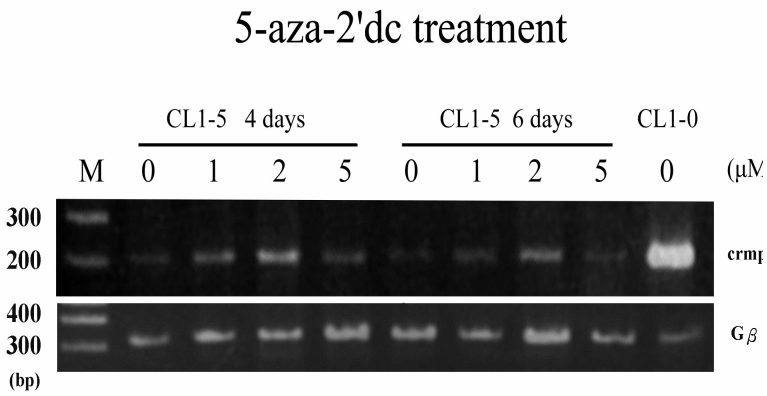


Fig 3B

IV. CRMP-1 expression is partially regulated by promoter methylation

CL1-5 was treated with 5aza-2'deoxycytidine (a demethylation drug). The expression of CRMP-1 was increased after two days treatment (Figure 4). But it declined after 5 days of treatment. Gbeta-like protein was an internal control.

Fig 4



四、參考文獻

1. Chu YW, Yang PC, Yang SC, Shyu YC, Hendrix MJC, Wu R, Wu CW: Selection of invasive and metastatic subpopulations from a human lung adenocarcinoma cell line. Am J Respir Cell Mol Biol 1997;17:353-60.
2. Chen JJW, Peck K, Hong TM, Yang SC, Sher YP, Shih JY, Wu R, Wu CW, Yang PC. Global analysis of gene expression in metastasis by a lung cancer model. Cancer Res 2001;61:5223-5230.
3. Shih JY, Yang SC, Hong TM, Yuan A, Chen JJ, Yu CJ, Chang YL, Lee YC, Peck K, Wu CW, Yang PC. Collapsin response mediator protein-1 and the invasion and metastasis of cancer cells. J Natl Cancer Inst 2001 ;93:1392-1400.
4. Shih JY, Lee YCG, Yang SC, Hong TM, Huang CYF, Yang PC. Collapsin response mediator protein-1: a novel invasion-suppressor gene. Clin Exp Metastas 2003;20:69-76.