

# 行政院國家科學委員會專題研究計畫 期中進度報告

## BCR-ABL 影響 CML 細胞移動之機制探討(2/3) 期中進度報告(完整版)

計畫類別：個別型  
計畫編號：NSC 95-3112-B-002-006-  
執行期間：95年05月01日至96年04月30日  
執行單位：國立臺灣大學醫學院生物化學暨分子生物學科暨研究所

計畫主持人：張智芬

報告附件：國外研究心得報告

處理方式：本計畫涉及專利或其他智慧財產權，1年後可公開查詢

中華民國 96年02月16日

## Introduction

Chronic myelogenous leukemia is caused by the t(9,22) (q34.1;q11.21) translocation that generates the BCR-ABL oncogene (3). P210<sup>BCR-ABL</sup> is an active tyrosine kinase, whose activity is responsible for its transformation activity (4). . CML myeloid cells circulate in large numbers in the blood at virtually all stages of differentiation, and it is clear that one of the defining characteristics of this disease is the uncoupling of differentiation from the ability to leave the BM (9). . A very recent study using human CD+34 progenitor cells isolated from human umbilical cord blood as a model system to express BCR-ABL, they have shown that BCR-ABL induced defects in progenitor adhesion to fibronectin and chemotaxis to SDF-1 $\alpha$ , which results are similar to that observed in progenitor cells isolated from the CML patients (16). In addition, BCR-ABL when expressed in different hematopoietic cell lines, Ba/F3, 32D and Mo7e, significantly impairs their response to SDF-1 in the transwell migration assay, which results are in accordance with what have been observed in isolated human CD34+ progenitor cells from CML patients (18, 19). Because SDF-1 $\alpha$  is one of the chemotactic agents that attract stem and progenitor cells to the BM space, the reduction of SDF-1 responsive migration by BCR-ABL is considered to contribute to the homing and retention defects typical of immature myeloid cells in CML. Herein, this project aims to understand how BCR-ABL relays signals to disturb normal actin organization, thus affecting migration behavior of the cells.

With the first year's effort, we have established that reduction cdc42 activity in K562 cells by inhibiting Bcr-abl kinase is responsible for restoring SDF-1-induced directional migration. In the 2<sup>nd</sup> year, we further showed that cdc42 activity was downregulated in CML cells isolated from patients with gleevec treatment, which was accompanied with an increased of directional migration. Incubation of cells with tat-cdc42V12 increased random migration but abolished gleevec-induced directional migration. By GST-Cdc42 pulldown experiment, we identified IQGAP as a major protein interacting with cdc42. Expression of IQGAP mutant defective in actin binding gave rise to similar contracting phenotype of gleevec-treated K562 cells. According to these results, our current hypothesis that BCR-ABL deregulates cdc42, masking chemotaxis-induced gradient activation of cdc42. As a result, IQGAP is randomly activated without a spatiotemporal control.

## **Materials and Methods**

### **Endogenous Rho GTPase activity assay**

The GST-RBD (RBD, RhoA-binding domain of Rhotekin) and GST-PBD (PBD, PAK Rac/Cdc42 binding domain) pull-down assays were used to detect cellular GTP bound RhoA and Rac1/Cdc42, respectively. In brief, cells were washed and lysed in a buffer containing 50 mM Tris-HCl, pH 7.2, 1% (v/v) Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 500 mM NaCl, 10 mM MgCl<sub>2</sub>, 1 mM PMSF and 100X dilution of protease inhibitor cocktail (Sigma). After centrifugation at 13,000 x g for 10 min at 4°C, the supernatants of the lysates were incubated at 4°C for 1.5 h with GST-RBD or GST-PBD-coupled glutathione-sepharose beads. The beads were washed four times with buffer containing 50 mM Tris-HCl, pH 7.2, 1% (v/v) Triton X-100, 150 mM NaCl, 10 mM MgCl<sub>2</sub>, 0.1 mM PMSF and 1000X dilution of protease inhibitor cocktail. The amounts of total and active, GTP-bound Rho GTPases were detected by Western blotting with a monoclonal antibody against RhoA (Santa Cruz Biotechnology), Rac1 (Upstate) and Cdc42 (Upstate).

### **Transwell chemotaxis assay**

The assay was performed in triplicate using 8 µm pore filter (Transwell, 24-well plate; Corning Costar, MA, U.S.A.). HL-60 cells or patient cells were preincubated in migration medium (serum-free RPMI 1640 medium) with or without 5 µM Gleevec for 17-18 h. Cells ( $10^5$ ) were then resuspended in 200 µl migration medium and placed in the upper chamber of the transwell. The lower chamber of the transwell was filled with 600 µl migration medium containing with or without hSDF-1 $\alpha$  (200 ng/ml). After 3 h of incubation, the upper wells were removed, and the migrating cells in the lower chamber were collected and manually counted with a hemocytometer. The percentage of migrating cells was calculated as: (number of migrating cells/ $10^5$ ) X 100% and the results were shown as mean  $\pm$  SD (n=3).

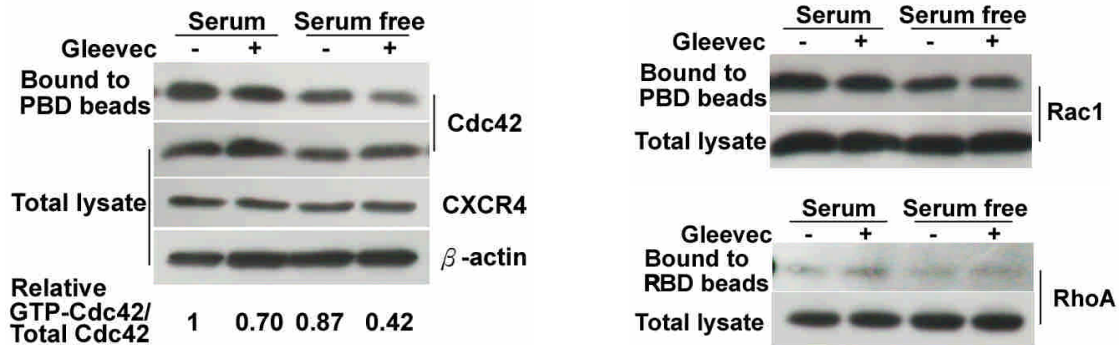
### **DIC (Differential Interference Contrast) microscopy for live cell imaging**

Live cell imaging was performed using MetaMorph 7.0 software to control a digital imaging system coupled to an inverted microscope (Axiovert 200M; Zeiss). Transmitted and fluorescent light illumination was controlled by built-in fluorescence shutter. A high-sensitivity CoolSNAP HQ CCD camera acquired images via the MetaMorph software. Live cell imaging cultured on

the POC-R open cultivation chamber (Zeiss) was performed with 100× DIC PlanApo oil objective (NA 1.3). Time lapse DIC video recordings were acquired every 20 sec for 15 min from the same field. Images presented are representative of independent experiments on different days.

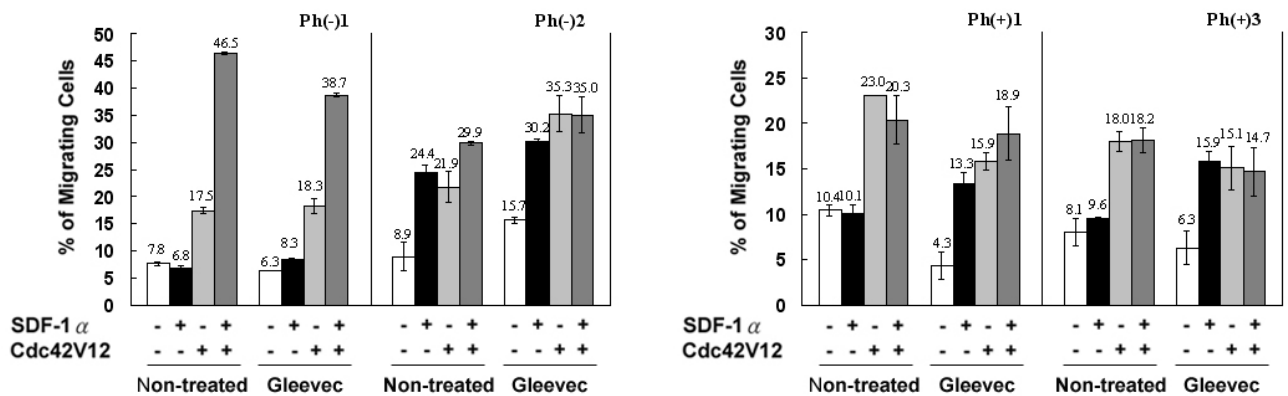
## Results

**Figure 1. Downregulation of cdc42 activity by gleevec treatment in myeloid cells isolated from CML patients.**



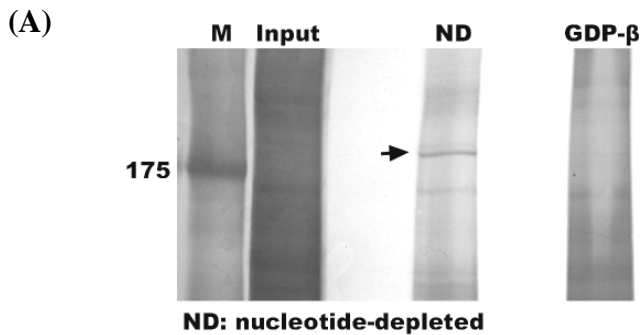
**Legend:** Fresh primary bone marrow samples were harvested from Ph(+)<sub>3</sub> patients and mononuclear cells were isolated. Primary cells maintained in serum-containing or -free RPMI 1640 medium were treated with or without Gleevec (5 μM) for 18 h. Cells were then harvested and lysed for Cdc42 (a), Rac1 (b), and RhoA (c) activity assays.

**Figure 2. Gleevec-induced SDF-1 responsive directional migration in Philadelphia positive CML cells was abolished by tat-cdc42V12 treatment.**

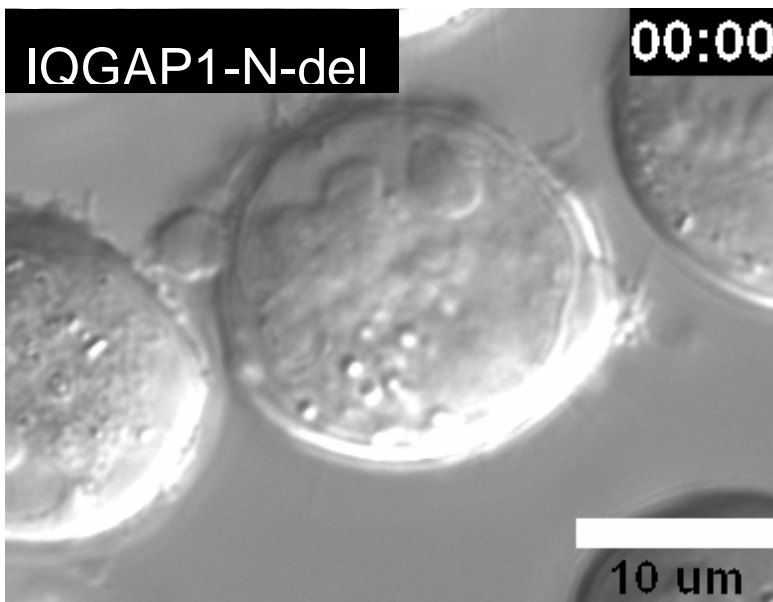


**Legend:** A total of 10<sup>5</sup> serum-free CML mononuclear cells were resuspended in fresh serum-free medium containing with or without Tat-Cdc42V12 (20 μg/ml) for 10 min and then added to the upper chamber of the transwell. The lower chamber of the transwell was filled with serum-free medium containing with or without hSDF-1α (200 ng/ml). After 3 h transmigrated cells recovered from the lower chamber were counted. The percentage of migration was calculated by considering the number of cells in the initial cell suspension (10<sup>5</sup>) as 100%. The data were the mean ± SD of three independent experiments.

**Figure 3. The involvement of IQGAP in gleevec-induced membrane blebbing in Gleevec-treated cells.**



(B)

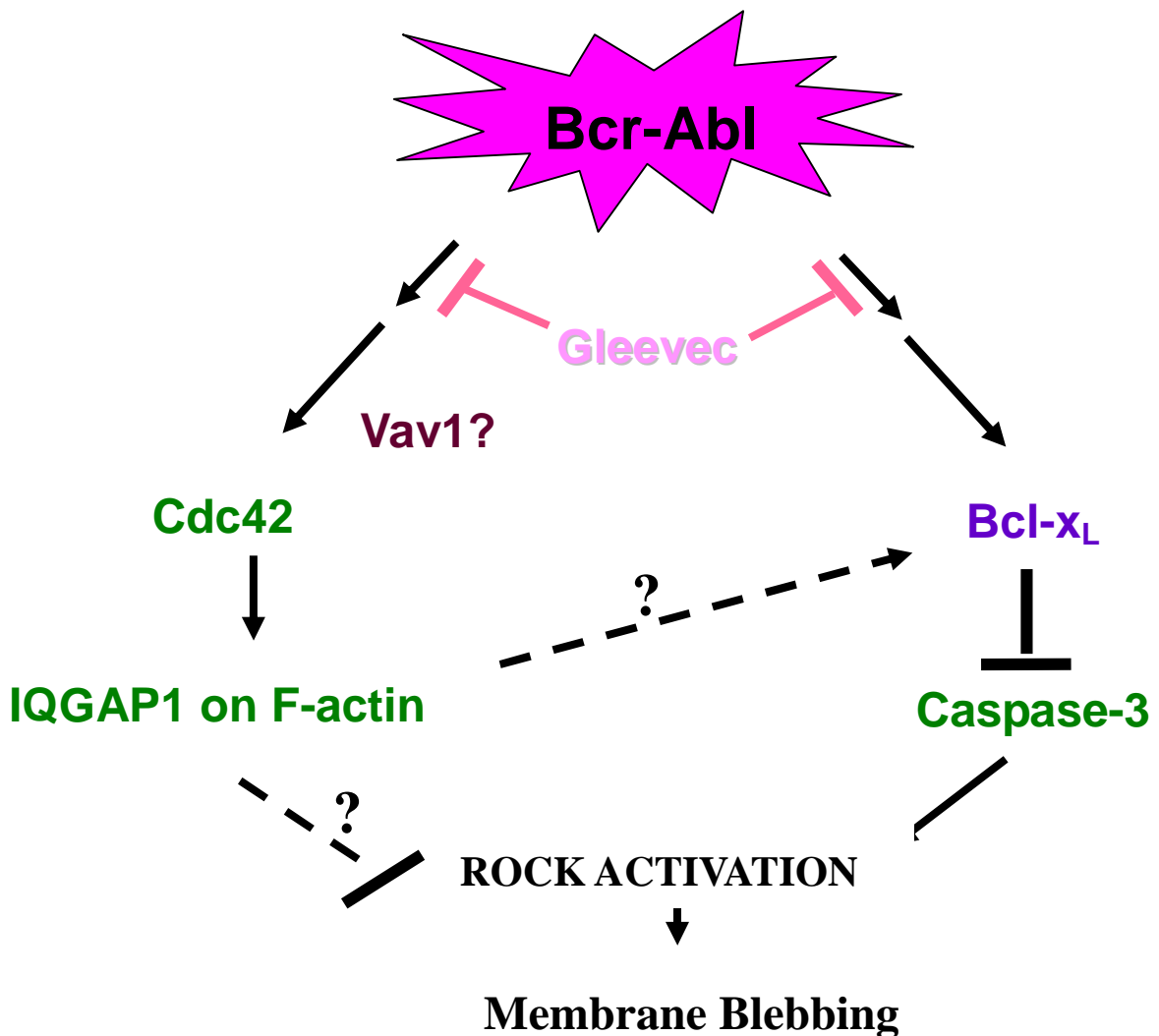


**Legend:** (A) Pull-down of proteins interacting with Cdc42 by GST-Cdc42 column. The pulled-down proteins were analyzed by SDS-PAGE and silver staining. One major protein indicated by silver staining was identified to be IQGAP by LC-MS/MS analysis. (B) K562 cells were transiently transfected with the wild-type IQGAP1 or its mutants (C-terminal only) and observed using time-lapse microscopy. These images were captured every 20 seconds for 15 min at 100X magnification, then processed and sequenced using Metamorph software.

## Discussion

This study showed that Cdc42-mediated signal has been deregulated in BCR-ABL transformed process. Too much cdc42 activation appears to disturb directional migration of CML cells. By GST-Cdc42 affinity capture, we found IQGAP is the major protein interacting with Cdc42 in the cells. Whether deregulation of IQGAP is responsible for the cytoskeletal changes in CML cells remains to be investigated. According to our results, the current hypothesis is shown in the diagram below.

### IQGAP1 on F-actin



### References.

1. Ptasznik, A., E. Urbanowska, et al. (2002). *J Exp Med* 196(5): 667-78.
2. Salgia, R., E. Quackenbush, et al. (1999). *Blood* 94(12): 4233-46

## 出席國際學術會議報告

計畫編號	NSC 95-3112-B-002-006
計畫名稱	BCR-ABL 影響 CML 細胞移動之機制探討
出國人員姓名 服務機關及職稱	台大醫學院生化暨分生所張智芬教授
出國時間地點	2006 年 8 月 17-20 日 瑞典
國際會議名稱	ICAP 2006 (International Conference on Arginine and Pyrimidines)

工作記要：

會議重要性：集合各國一流大學研究所學者共同交換研究學習心得

與會經過：

ICAP 2006 (International Conference on Arginine and Pyrimidines)於 2006 年 8 月 17-20 日在瑞典,Lund University 舉行。

會議中有四個 Sessions 分別是

1. ARGININE AND PYRIMIDINES
2. DEOXYNUCLEOSIDE KINASES AND RELATED ENZYMES I
3. STRUCTURE-FUNCTION OF SALVAGE, DEAMINASE, SYNTHASE ENZYMES
4. NUCLEOTIDE/NUCLEOTIDE POOLS

本人受邀於該會發表 plenary lecture，題目為 Mitotic Control of dTTP Pool by Anaphase Promoting Complex/Cyclosome。

與會心得：

此次會議涵蓋主題十分廣泛，議程十分緊湊，從早上八點三十分至下午六點均有相當精彩的演講，此會議已有四十年度歷史，每二年輪流在歐洲不同研究單位，該與會學者多是研究核酸代謝的專家，而結構生物學及蛋白化學為本會的主軸，在會議中，我學習到許多新的核酸代謝的知識，尤其是核甘酸相似藥物在研究這些酵素的相關應用。雖然本人多年來專注於 Thymidine Kinase 1 的調節分子機構，然對於該蛋白分子參與核酸新陳代謝的角色，並不全然了解。

此次是第一次參與該會議並與多位參與 TK1 蛋白結構分析的研究人員有深入的交談，獲益良多。另外，在會議當中與 Nobel Laureate William Lipscomb (Harvard University)交談用餐，這位在生化蛋白科學有巨大貢獻的年邁學者，其謙遜過良的治學態度令人敬佩，同時 Dr. Peter Reichard, a previous Nobel faculty 特別和我交談數次，針對有關 dTTP pool 的調節，有深入意見交換，在本會我的演講為時 50 分鐘，得到相當大的鼓勵。

攜回資料名稱及內容簡要描述：

ICAP 2006 會議摘要 乙冊