行政院國家科學委員會專題研究計畫　成果報告

探討 ZAK 結合蛋白在 ZAK 引起心肌肥大時的功能

計畫類別：個別型計畫
計畫編號：NSC93-2311-B-040-008-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：中山醫學大學口腔醫學研究所

計畫主持人：楊肇基

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 10 月 31 日

ZAK is able to stimulate cardiac hypertrophy. It might lead to the organization of myofibrils into sacomeric units. As shown in figure 1, expression both of ZAK and ZAKE/E significantly induced the organization of sacromere structure as compare to control or ZAKdn cells.

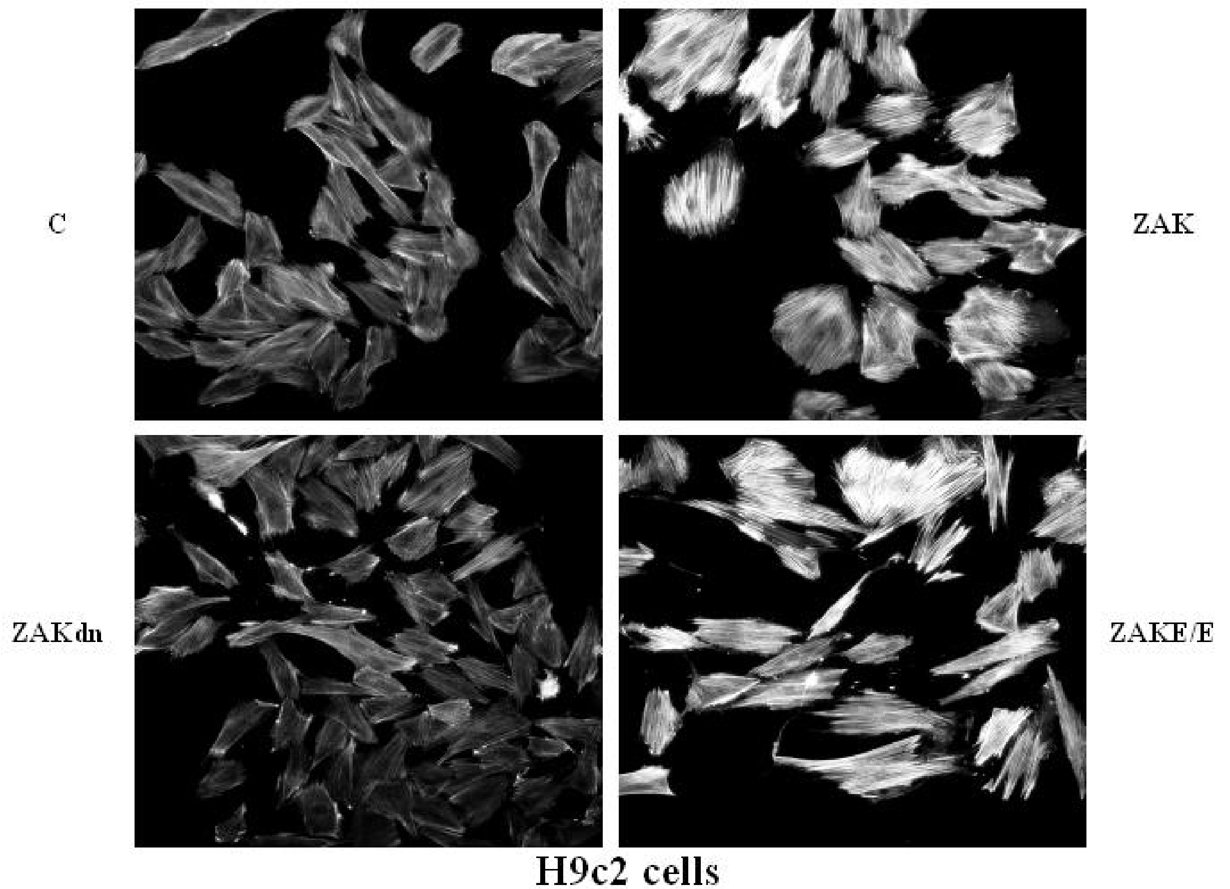


Fig. 1

These experiments clearly demonstrated that the expression ZAK in cardiac myocyte could induce cheracteric features of cardiac hypertrophy in cultured myocytes.

Beside the ZZaPK gene was identified to be associated with ZAK, there are 13 different genes that might be ZAK associated (fig. 2 and Table 1). These genes can be

category into three groups, base on their activity. First of these groups is genes that might be transcription factors, ZZaPK, RLF, ZAP36 (similar to ZXDA), and ZAP73 (Zn finger protein). Among of these genes, ZAP36 and ZAP 73 genes are novel genes. Second group of these genes is involved in sacromere structure, ARGBP2, CH-TOG, Tropomyosin isoform, Nexilin, ARHGDIB(RhoGDI), and myosin binding protein C. The third gene is RPS20(ribosomal). Since the characteristic features of hypertrophy include an increase in cell size, elevated expression of atrial natriuretic factor, exit the cell cycle, induction of sarcomere organization, and increasing protein synthesis. These ZAK associated genes might indicate that they might play as intermediates for ZAK function to induce cardiac hypertrophy.

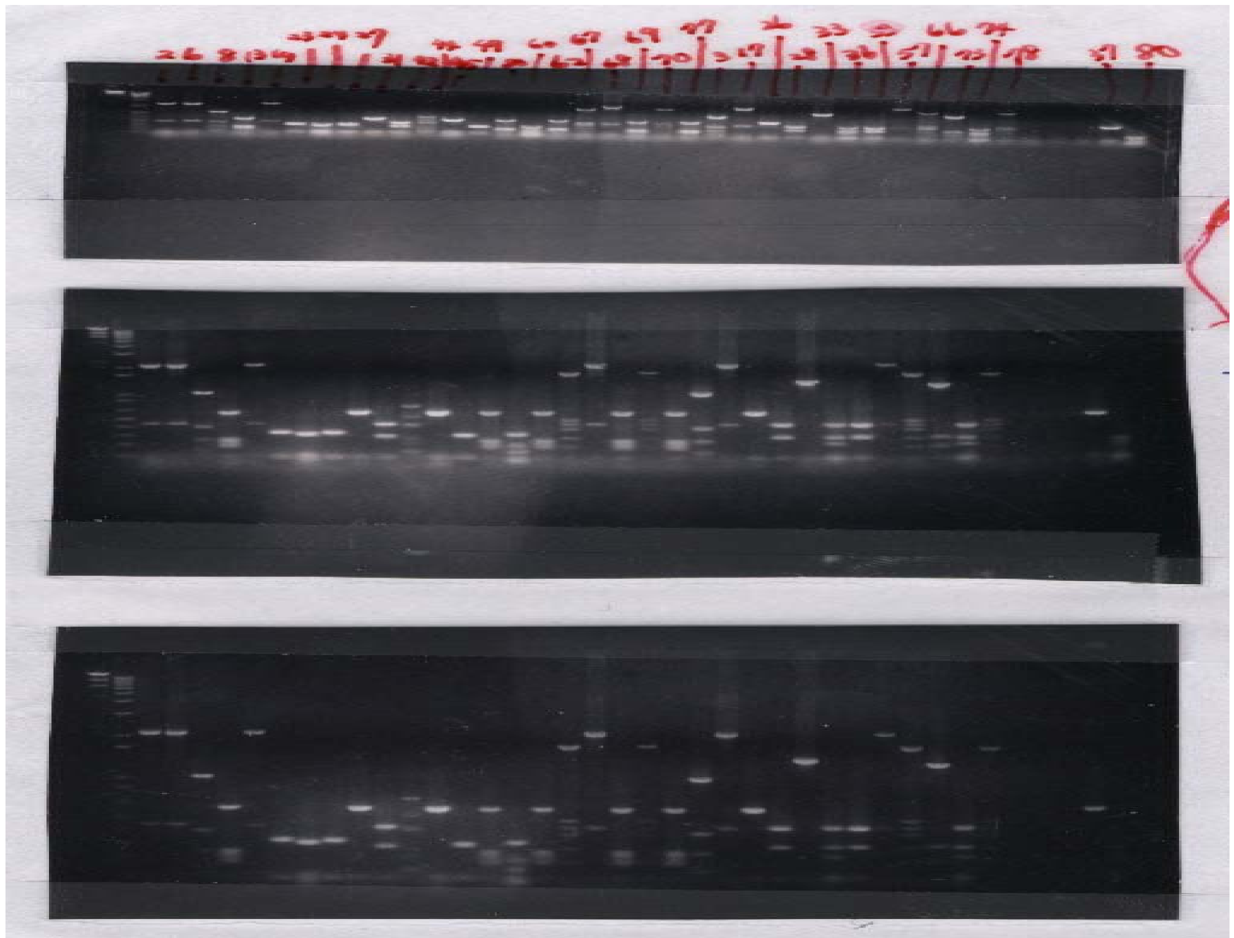


Fig. 2

TABLE 1

Clone	size	Gene
2,6,14,68,17,57	2.9K	ARGBP2
8,3	2.0K	RLF(Zn finger protein)
23,27	0.65K	RPS20(ribosomal)
13,50,62,69,77	1.7K	
24,49	0.85K	CH-TOG
31,45,26,37	0.9K	Tropomyosin isoform
42,28,53	1.0K	Nexilin
44	1.0K	ARCN1
60	1.3K	ARHGDIB(RhoGDI)
67,70,66,78	3.8K	ZZaPK
33	1.6K	ZZaPK
36,74	1.6K	similar to ZXDA

73 2.3K	Zn finger protein
80 2.9K	myosin binding protein C

The human RhoGDI gene was cloned, based on the sequenced published in Genbank, using the RT-PCR method and the gene was subcloned into a tetracycline inducible mammalian expression vector, pTRE2, to generate pTRE2-RhoGDI. The inducible RhoGDI expressing cells were selected. It is interesting to find that RhoGDI is able to stimulate cardiac hypertrophy and lead to the organization of myofibrils into sacomeric units (Fig. 3). Carefully comparison the hypertrophy induced by ZAK and RhoGDI. It is discovered that both ZAK and RhoGDI are able to induce cardiac hypertrophy and then RhoGDI induces larger cell size than ZAK does. Moreover, RhoGDI cells lost membrane integrity. The relationship between ZAK and RhoGDI to induce cardiac hypertrophy will be carried out in the furture.

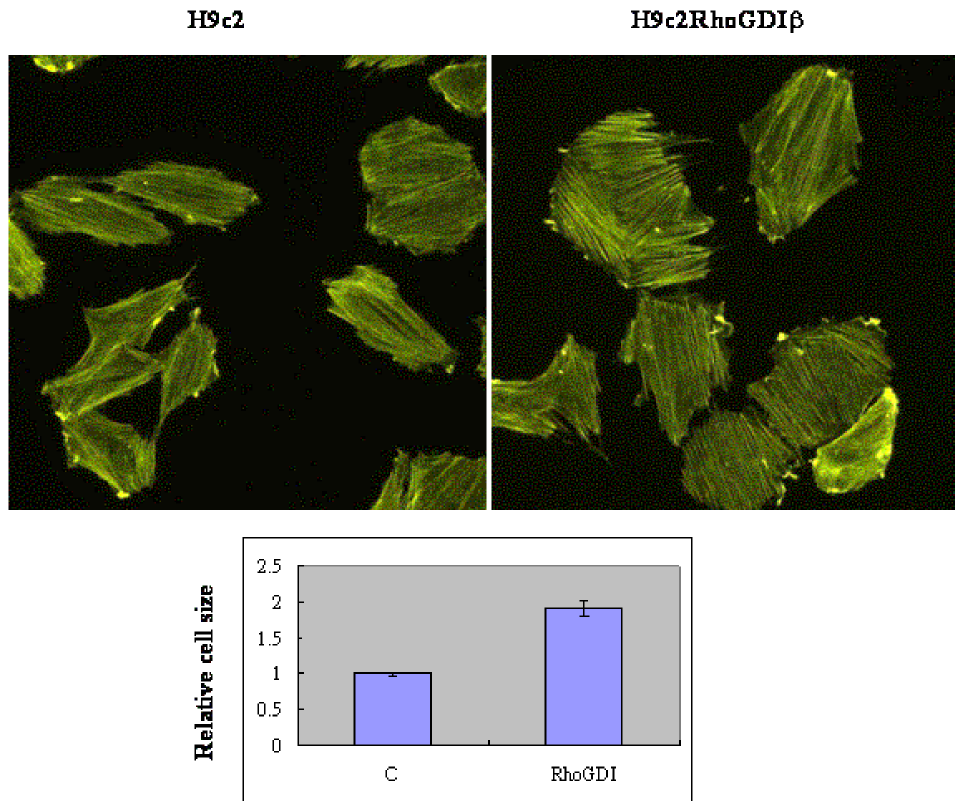


Fig. 3

The yeast-two hybrid experiment demonstrated that the ZAK is able to associate with RhoGDI. To examine whether ZAK interacts *in vitro* with GST-RhoGDI, GFP-tagged ZAK was pull-down with GST-tagged RhoGDI. As shown, GST-tagged RhoGDI is able to associate with GFP-tagged ZAK independent of the intrinsic kinase activity of ZAK (Fig. 4).

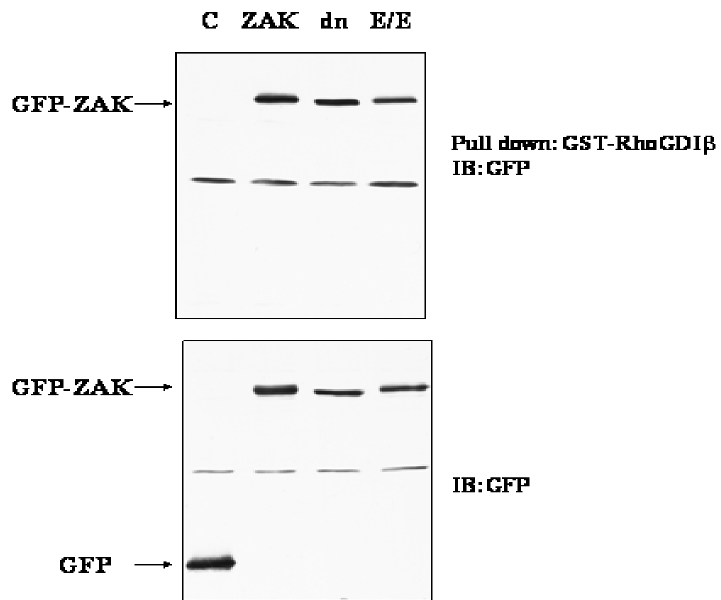


Fig. 4

H9c2 cells underwent hypertrophy growth when RhoGDI is expressed. To determine whether the effects of RhoGDI on hypertrophy growth might influence on their ability to proliferate in H9c2 cardiac cells. We measure the growth rate of cells expressing mock and RhoGDI. The RhoGDI expressing cells showed decreased growth rate in comparison with parental cells. To determine the cell cycle in which RhoGDI expressed cells grew slower than the parental, asynchronous cultures were trypsinized and their cell cycle distributions were analyzed using flow cytometry. RhoGDI -expressed cells had a significantly higher percentage of cells in the G_0/ G_1 phase of the cell cycle stage compared

with the control cells (Fig. 5).

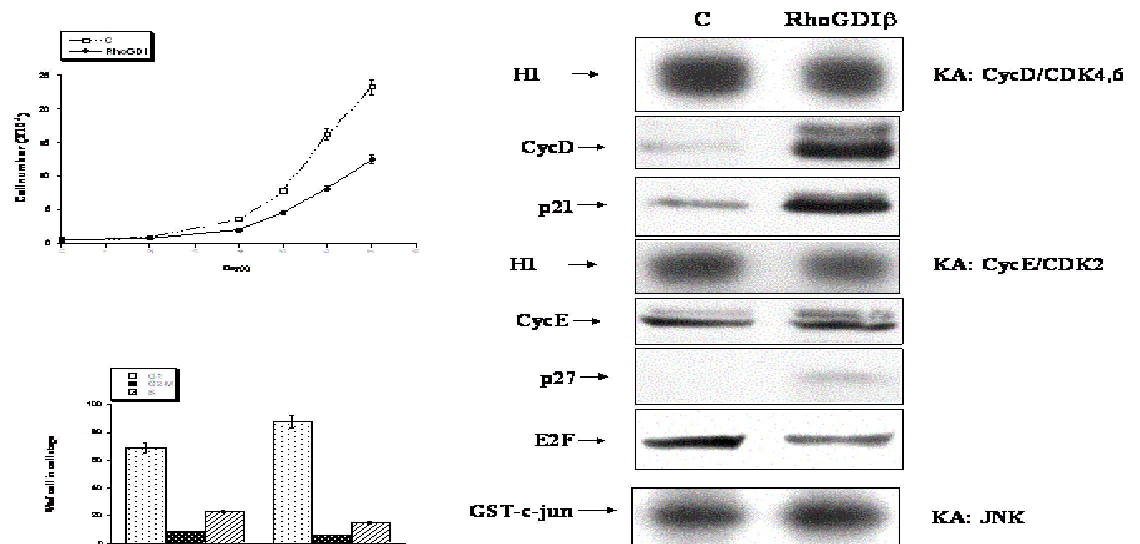


Fig. 5

To determine the functions of RhoGDI might regulate the activation of Rho family GTPases (Rho, Rac, and CDC42) in H9c2 cardiac cells. We measure the subcellular localization of these Rho GTPases in cells expressing mock and RhoGDI. The RhoGDI expressing cells showed increased Rac but not Rho and CDC42 protein levels in membrane fraction as well as in the cytosolic fraction in comparison with parental cells Fig. 6C). These results suggested that RhoGDI might play a role that regulates the expression levels of Rac. We, therefore, examined the total mRNA and protein levels of Rac in RhoGDI expressing cell, the results present here indicated that RhoGDI might stimulate transcriptional

levels of Rac mRNA (Fig. 6A and B).

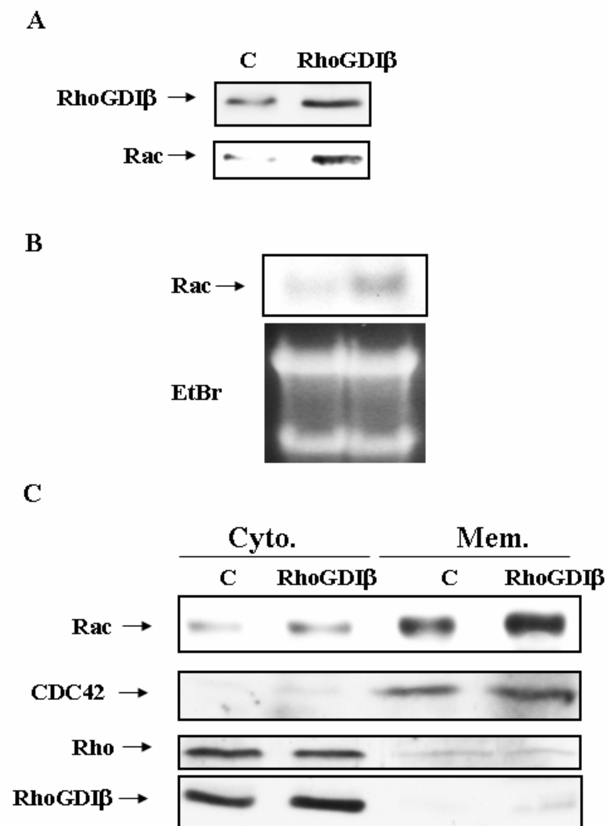
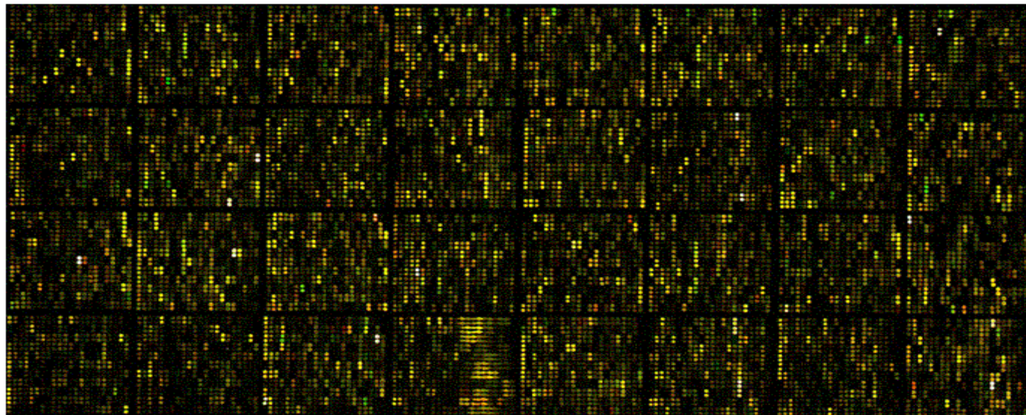


Fig 6

The RhoGDI expressed cardiac myocytes were used to screen cDNA CHIP. RhoGDI is able to onset the cardiac hypertrophy and it might regulate several genes that are relative to the cardiac hypertrophy. A variety of genes are either up-regulated or down-regulated in the study (Fig.7). These genes will subject to analysis in the future study.

H9c2 V.S. H9c2RhoGDIβ



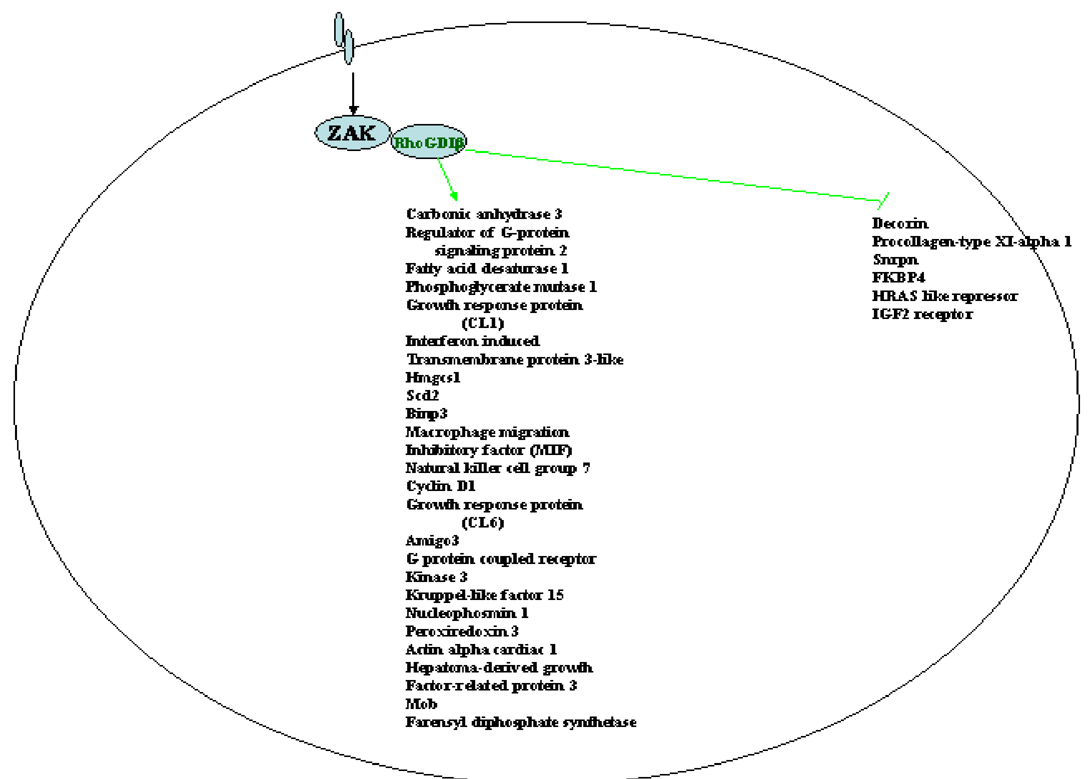


Fig. 7

Two of these ZAK associated proteins, similar to ZXDA and ZAP73, are turn out to be novel. To proceed the cloning the gene, ZAP73 cDNA, from the yeast

two-hybrid screening was used to screen human plancenta cDNA library. The full-length was cloned and the gene is belonging to a novel Krüppel-type C2H2 zinc finger member (Fig. 8). The function of this gene is needed to be carefully study in the near furture.

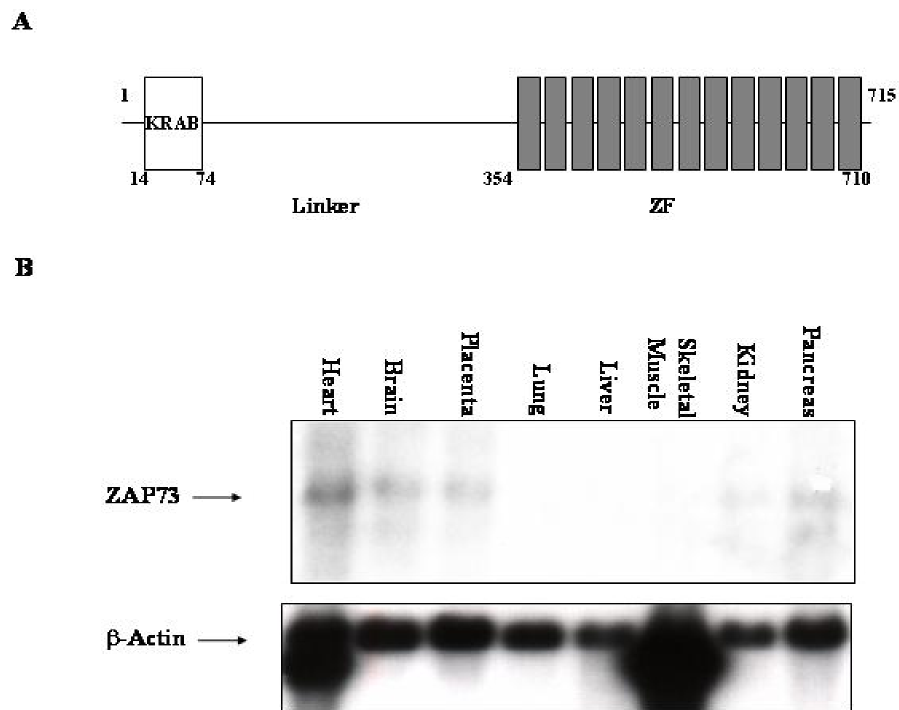


Fig. 8

The human ArgBP2 gene was interesting and cDNA cloned, based on the sequenced published in Genbank, using the RT-PCR method. ArgBP2 is an SH3- and SoHo- domain containing protein. The yeast-two hybrid experiment demonstrated that the ZAK is able to associate with ArgBP2. To examine whether ZAK interacts *in vivo* with ArgBP2, GFP-tagged ZAK cDNA was

co-transfected with FLAG-tagged ArgBP2 cDNA into 293T cells. As shown, FLAG-tagged ArgBP2 was able to associate with GFP-tagged ZAK independent of the intrinsic kinase activity of ZAK (Fig. 9).

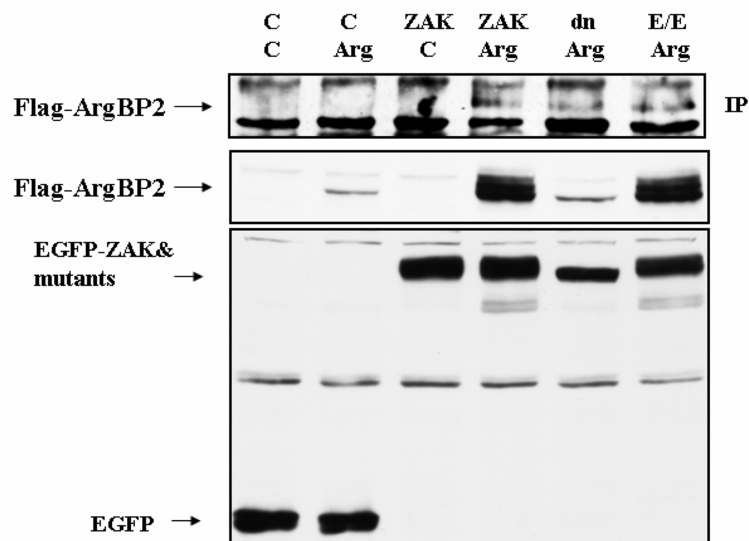


Fig 9

The over-expression of ZAK and ArgBP2 in 293T cells showed ArgBP2 mobility retardation in SDS-PAGE. The *in vitro* treatment of FLAG-ArgBp2 with calf intestine alkaline phosphotase increased its mobility in SDS-PAGE (Fig. 10). Thus ArgBP2 seems to be able to undergo phosphorylation dependent the activities of ZAK. This result indicated that the ArgBP2 phosphorylation is

required for the existence of kinase activity within ZAK.

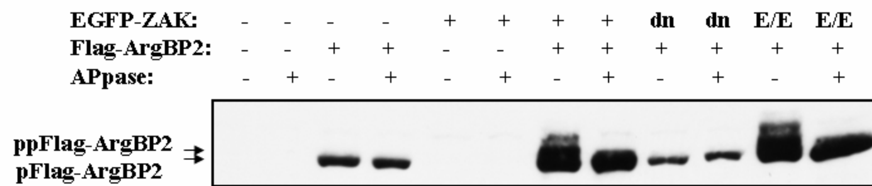


Fig 10

It has been indicated that the multi-adaptor protein ArgBP2 links to the E3 ubiquitin ligase Cbl. We determine that ArgBP2 might link ZAK and Cbl in a signaling complex in mammalian cells. The result present here suggests that ZAK, ArgBP2, and Cbl form a tertiary signaling complex (ZAK-ArgBP2-Cbl) (Fig. 11)

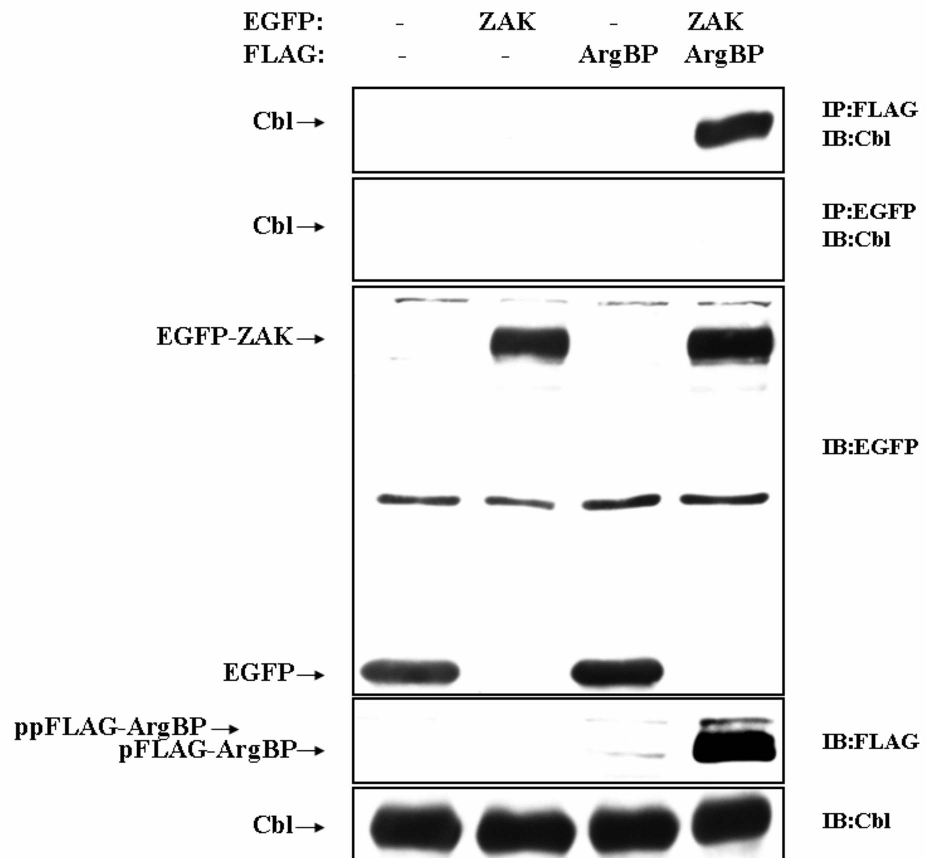


Fig. 11

In this regard, the primary goal of this project is to study on these ZAK associated protein (RhoGDI) that might play the role on the development of cardiac hypertrophy and the functional significance of the interactions between ZAK, ArgBP2, and Cbl and in particular their role in signaling complex.