# 行政院國家科學委員會專題研究計畫　成果報告

## TGF-Β對於肝癌細胞死亡的調控與機制之研究(5/5)

計畫類別：個別型計畫
計畫編號：NSC92-2321-B-002-005-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：國立臺灣大學醫學院分子醫學研究所

計畫主持人：陳瑞華

計畫參與人員：陳瑞華、郭津岑、王琬菁、陳奕如

報告類型：完整報告
報告附件：出席國際會議研究心得報告及發表論文
處理方式：本計畫可公開查詢

中　華　民　國 93 年 12 月 13 日

# 國科會生物處尖端科學研究計畫執行成果摘要報告表

計畫主持人：陳瑞華

計畫名稱：Mechanism and regulation of TGF-β-induced apoptosis in human hepatoma cells.

執行期限：88/2/1 - 93/7/31

## 一、 全程計畫研究內容及成果摘要：（簡述全程計畫目標、研究內容及研究成果，以A4格式撰寫1-2頁為原則）

The overall goal of this proposal is to dissect the signaling pathway of TGF-β-induced apoptosis and to elucidate the mechanism of cross-talk between this apoptotic pathway and anti-apoptotic pathways induced by several growth factors and cytokines. During the five-year funding period, we have not only accomplished various Specific Aims listed in the proposal, but also established new research areas in the laboratory. Our major achievements are listed below:

**1. Identification and characterization of cross-talk between the pro-survival IL-6 pathway and pro-apoptotic TGF-β pathway.**

Through collaboration with Dr. Min-Liang Kuo at National Taiwan University, we found IL-6 efficiently inhibits the apoptotic effect of TGF-β, but not its anti-proliferative effect. Subsequent mechanistic study indicates that both PI 3-kinase/Akt pathway and JAK/STAT3 pathway are responsible for the anti-apoptotic role of IL-6, with the former playing a greater role. These findings are published in JBC and the paper has been well cited since its publication (>50 times).

**2. Identification of DAPK as an apoptotic effector of TGF-β.**

To systematically investigate the apoptotic mechanism of TGF-β, we performed cDNA microarray analyses to identify genes whose RNA levels altered during TGF-β-induced apoptosis. This led to the identification of DAPK, an pro-apoptotic kinase, as a TGF-β-inducible gene. We show that the promoter of DAPK is activated by TGF-β through the action Smad2, Smad3 and Smad4. Furthermore, overexpression of DAPK triggers apoptosis in the absence of TGF-β, whereas inhibition of DAPK activity protects cells from TGF-β-induced apoptosis, blocks TGF-β-induced release of cytochrome c from mitochondria and prevents TGF-β-induced dissipation of the mitochondrial membrane potential. Our findings indicate that DAPK mediates TGF-β-dependent apoptosis by linking Smads to mitochondrial-based pro-apoptotic events. This study reveals the apoptotic mechanism of TGF-β and is published in Nature Cell Biology, the leading journal in the cell biology field.

**3. Unraveling the pro-apoptotic mechanism of DAPK.**

The identification of DAPK as an apoptotic effector of TGF-β has opened a new avenue of our research. We were particularly interested in the underlying mechanism of this well-documented pro-apoptotic kinase. We found that DAPK suppresses integrin activity through an inside-out mechanism, thereby leading to the attenuation of cell adhesion. As a consequence, DAPK blocks integrin-mediated matrix survival signals, upregulates p53 protein level, and induces an anoikis-type apoptosis. This study not only solves a long-standing mystery on the apoptotic mechanism of DAPK, but also implicates a cytoskeketon effect of this molecule. The identification of DAPK as an anoikis inducer has drawn a great attention in the field of cell biology. Therefore, our paper, when published

in J Cell Biology, was chosen as a highlight in that issue.

**4. Identification of DAPK as a potent regulator of actin cytoskeleton.**

In searching for the potential substrates of DAPK, we identified myosin light chain (MLC) as a DAPK substrate both in vitro and in vivo. Through phosphorylating MLC at serine 19, DAPK promotes the formation and assembly of stress fibers. However, this stress fiber induction effect of DAPK does not cause a concomitant stimulation of focal adhesion assembly. On the contrary, DAPK negatively regulates focal adhesion formation. Therefore, these results identify a novel and unique function of DAPK in the uncoupling of stress fibers and focal adhesions. Such uncoupling would lead to a perturbation of the balance between contractile and adhesion forces and subsequent cell detachment, which might contribute to its pro-apoptotic activity. Together with the study described in "3", our research demonstrates the novel biological function of DAPK in regulating actin cytoskeleton.

**5. Dissecting the signaling cross-talk between DAPK and ERK.**

We identified the extracellular signal-regulated kinase (ERK) as a DAPK-interacting protein. DAPK interacts with ERK through a docking sequence within its death domain and is a substrate of ERK. Phosphorylation of DAPK at Ser 735 by ERK increases the catalytic activity of DAPK both in vitro and in vivo. Conversely, DAPK promotes the cytoplasmic retention of ERK, thereby inhibiting ERK signaling in the nucleus. This reciprocal regulation between DAPK and ERK constitutes a positive feedback loop that ultimately promotes the apoptotic activity of DAPK. Disruption of this regulatory circuit by blocking ERK-DAPK interaction attenuates DAPK-mediated apoptosis, whereas downregulation of the endogenous DAPK blocks ERK-mediated apoptosis. These results indicate that bi-directional signaling between DAPK and ERK may contribute to the apoptosis-promoting function of the death domain of DAPK. This study has been submitted to EMBO J. and is currently under revision.

**6. Studies on oncogenic tyrosine kinases.**

In addition to apoptosis-related research, another focus of our laboratory is the oncogenic tyrosine kinases. Although this topic is not included in the original grant proposal, it has indeed been supported by the Frontier grant, my only funding source. Within this five-year period, we have researched into two kinases, Etk and Brk. We found Etk mediates the cell transformation signal of oncogene Src to the STAT3 activation. This finding provides a mechanistic explanation of Src-induced STAT3 activation and is of significance in the molecular aspect of tumor biology. In the study of Brk (breast tumor kinase), we found Brk specifically phosphorylates paxillin at Y31 and Y118. Through this phosphorylation, Brk promotes Rac-1 activation, cell migration and invasion. Since the original identification of Brk in the metastatic breast tumors, our findings provide the first molecular link between Brk and metastatic malignancy. Both ERK and Brk studies are published in Mol Cell Biol.