

行政院國家科學委員會專題研究計畫成果報告

Bcl-2 與 Bax 基因表現對胃癌抗藥性之影響

與其相關機制之研究

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主持人：鄭安理 國立台灣大學醫學院 內科

一、中文摘要：

「細胞凋亡」(apoptosis)是化學治療藥物引發癌細胞死亡之主要機制。Bcl-2 與 Bax 是主宰細胞凋亡的兩個重要基因。Bcl-2 蛋白的表現，可「抵抗」細胞凋亡；相反的，Bax 則可「促進」細胞凋亡。過去幾年來，我們一直致力於提昇胃癌化療的成績，並闡明胃癌細胞產生抗藥性的原因，在本研究中，我們進一步探討 Bcl-2/Bax 在胃癌的化學治療抗藥性中所扮演的角色。

臨床化療反應與組織染色之關聯研究：運用免疫組織化學染色法進行 29 位胃癌病患胃癌組織之 *Bcl-2* 與 *Bax* 蛋白表現之測定，再與化學治療反應進行關聯性分析。其中分別有 3 位與 21 位胃癌病患組織有 *Bcl-2* 與 *Bax* 蛋白表現(分別佔 $10.3 \pm 5.7\%$ 與 $72.4 \pm 8.3\%$)，但與胃癌化學治療反應並無統計上有意義之關聯(P 值分別為 0.078 與 0.943)。

胃癌細胞株研究：以 Bcl-2 轉殖進入原先並無 Bcl-2 表現之胃癌細胞株(AGS; NCI-N87)中，取得 Bcl-2 之穩定轉殖株，再比較轉殖前後細胞株各種抗癌藥物 IC_{50} 之變化。發現 Bcl-2 的「高度表現」可造成胃癌細胞全面多重藥物抗藥性。此種 Bcl-2 轉殖胃癌細胞株將可作為未來「篩選」「反轉 Bcl-2 抗藥性藥劑」之體外研究模式。

結論：本研究結果顯示 Bax 在胃癌組織中之表現較 Bcl-2 常見，但在臨床化療反應與組織染色之關聯研究中，兩者與胃癌化學治療反應均未達統計上有意義之關

聯。然而，在胃癌細胞株研究中，Bcl-2 的「高度表現」顯示與胃癌細胞之化學藥物全面抗藥性相關。這些結果有助於了解胃癌細胞之抗藥性及抗凋亡性，作為將來提昇胃癌化療效果之參考依據。

關鍵詞：Bcl-2，Bax，胃癌，化療抗藥性，細胞凋亡

Abstract

“Apoptosis” is the final common pathway of most chemotherapy-induced tumor cell death. Bcl-2 and Bax are two of the key players regulating the apoptosis phenomenon. Bcl-2 is a blocker of apoptosis, while Bax functions as a promoter of apoptosis. In this study, we examined the roles of Bcl-2 and Bax in drug resistance of gastric cancer cells.

Bcl-2 and *Bax* protein expression were determined by immunohistochemical stains in 29 gastric cancer tissues, and correlated with chemotherapy responses (13 responders and 16 non-responders). *Bcl-2* and *Bax* protein expression was found in $10.3 \pm 5.7\%$ and $72.4 \pm 8.3\%$ gastric cancer tissues, respectively. However, In this group of patients, we cannot find statistically significant correlation between *Bcl-2* or *Bax* protein expression and chemotherapy responses ($P=0.078$ and 0.943 by Fisher exact test, respectively).

In cell line studies, we have examined 7 human gastric cancer cell lines. All of them had Bax expression, and 5 of them had Bcl-2 expression (except AGS and NCI-N87). These two Bcl-2(-) parental gastric cancer

lines were transfected with Bcl-2 cDNA expression vector, selected for stably transfected clones, verified by Western blot, then selected for further study. Compared with the parental cell lines, the Bcl-2 transfectants had 1.7- to 125-fold higher IC₅₀ of multiple structurally unrelated chemotherapeutic agents, including 5-FU, cisplatin, doxorubicin, etoposide, paclitaxel, and docetaxel.

We concluded that (1) *Bax* protein is more frequently expressed than *Bcl-2* in gastric cancer tissues; (2) although we cannot find statistically significant correlation between *Bcl-2* or *Bax* protein expression and HDFL-based chemotherapy responses, our *in vitro* cell line model suggests that Bcl-2 overexpression may be a multidrug resistance marker of gastric cancer. This *in vitro* model is potentially useful for searching of Bcl-2 modulating agents, which may help reverse drug resistance conferred by Bcl-2 overexpression.

Keywords: Bcl-2, Bax, gastric cancer, drug resistance, apoptosis

二、緣由與目的:

“Apoptosis” is the final common pathway of most chemotherapy-induced tumor cell death^[1]. “Drug resistance” is considered a form of “apoptosis resistance”^[1,2]. Bcl-2 and Bax are two of the key players regulating the apoptosis phenomenon. Bcl-2 is a blocker of apoptosis, while Bax functions as a promoter of apoptosis. The Bcl-2 gene was first discovered because of its involvement in the t(14;18) chromosomal translocations commonly found in low-grade follicular lymphomas, which result in deregulation of bcl-2 gene expression and cause inappropriately high levels of Bcl-2 production^[3,4]. Several mammalian homologs of Bcl-2 have recently been discovered, some of which function as inhibitors of apoptosis (Mcl-1, A1, Bcl-X_L)^[5-7] and others as promoters of apoptosis (Bax,

Bad, Bak, Bcl-X_S)^[8-10] that oppose the actions of Bcl-2 protein. Several studies have shown Bcl-2 protein correlates with relative resistance to a wide spectrum of anti-cancer drugs in lymphoma and leukemia cells^[11-13].

Gastric cancer is moderately sensitive to chemotherapy. In the past few years, we have been working on improving the results of systemic chemotherapy for gastric cancer, and clarifying the mechanisms which may contribute to the drug resistance of gastric cancer. We have already completed three prospective clinical trials of combination chemotherapy for gastric cancers. The results have been encouraging^[14-16]. Although the response rate has been greatly improved, the duration of remission was still short, and hence the overall survival was not remarkably improved. The reason for the short response duration of most gastric cancer patients was most likely due to the emergence of drug resistance. In our prior studies, we have already studied the associations of the expression of thymidylate synthase^[17,18], multidrug resistance-1^[17,19], multidrug resistance-associated protein^[17], glutathione S-transferase- π , p53 over-expression^[20], and the extent of apoptosis^[21] with drug resistance of gastric cancers. In this study, further examined the roles of Bcl-2 and Bax in drug resistance of gastric cancer cells.

The anti-apoptosis function of Bcl-2 can function both as an ion channel and as an adaptor (or docking) protein^[22]. As an ion channel, Bcl-2 may block the release of cytochrome c from mitochondria, and therefore may inhibit the activation of caspase-3^[23,24]. As an adaptor protein, many Bcl-2 family proteins can interact through formation of homotypic and heterotypic dimers, such as Bax/Bcl-2 heterodimer^[8]; in addition, several non-homologous proteins were found to be able to bind with bcl-2 and modulate apoptosis^[22]. Among them, the protein kinase Raf-1^[25,26], the protein phosphatase calcineurin^[27], the p53-binding

protein p53-BP2^[28], and several proteins with unknown functions, such as CED-4^[29], BAG-1^[30], etc. In contrast, the pro-apoptotic protein Bax apparently does not interact with these non-homologous proteins, suggesting the central role of Bcl-2 in the control of apoptosis^[22].

There are several possible hypotheses for the possible mechanisms of Bcl-2-conferred drug resistance in gastric cancer, if it does exist. *First*, Bcl-2 may form heterodimer with Bax^[8], and may neutralize the pro-apoptotic function of Bax. *Second*, Bcl-2 may inhibit caspase via interaction through CED-4. Caspase is the key enzyme in the final pathway of apoptosis. Bcl-2, CED-4, and caspase may bind together^[29], and thus Bcl-2 potentially may regulate caspase through the binding. *Third*, Bcl-2 may cause a higher or more sustained degree of NF- κ B activation. Recent studies have shown that the activation of the NF- κ B may protect cells from apoptosis^[31]. We have also demonstrated that almost all anti-cancer drugs are able to induce NF- κ B activation in most representative cancer cells^[32]. Bcl-2 was recently found to be able to activate NF- κ B via calcineurin, in which Bcl-2 might bind with calcineurin-NF-AT system^[27], and NF-AT might interact with NF- κ B^[33].

三、結果與討論:

In this study, *Bcl-2* and *Bax* protein expression were determined by immunohistochemical stains in 29 gastric cancer tissues, and correlated with chemotherapy responses. *Bcl-2* and *Bax* protein expression was found in $10.3 \pm 5.7\%$ and $72.4 \pm 8.3\%$ gastric cancer tissues, respectively. Among them, there were 13 responders and 16 non-responders to HDFL (weekly 24-hour infusion of high-dose 5-FU and leucovorin)-based chemotherapy. For *Bcl-2* expression, 3 of 3 patients with *Bcl-2*(+) responded to chemotherapy as 10 of 26 patients with *Bcl-2*(-) did ($P = 0.078$, Fisher

exact test). For *Bax* expression, 10 of 21 patients with *Bax*(+) responded to chemotherapy as 3 of 8 patients with *Bax*(-) did ($P = 0.943$, Fisher exact test). In this group of patients, we cannot find statistically significant correlation between *Bcl-2* or *Bax* protein expression and HDFL-based chemotherapy responses.

In cell line studies, we have examined 7 human gastric cancer cell lines, including AGS, NCI-N87, KATO-III, RF-1, PF-48, SNU-1, SNU-16. All cell lines had Bax expression, and 5 of them had Bcl-2 expression (except AGS and NCI-N87). These two Bcl-2(-) parental gastric cancer lines were transfected by the method of calcium phosphate precipitation and G418 selection. The Bcl-2 cDNA sequences were expressed by SV40 enhancer/promoter regulatory element (pC Δ j-Bcl-2). Four stably trans-fected clones AGS-3, AGS-18, N87-5, N87-10, which had stable Bcl-2 protein overexpression, as verified by Western blot, were selected for further study. There were no significant differences in growth rate and cell cycle fractions between the parental cell lines and the Bcl-2 transfectants. The IC₅₀ of anti-cancer drugs was determined by MTT assay. Compared with the parental cell lines, the Bcl-2 transfectants had 1.7- to 125-fold higher IC₅₀ of multiple structurally unrelated chemotherapeutic agents, including 5-FU, cisplatin, doxorubicin, etoposide, paclitaxel, and docetaxel.

We concluded that (1) *Bax* protein is more frequently expressed than *Bcl-2* in gastric cancer tissues; (2) although we cannot find statistically significant correlation between *Bcl-2* or *Bax* protein expression and HDFL-based chemotherapy responses, our *in vitro* cell line model suggests that Bcl-2 overexpression may be a multidrug resistance marker of gastric cancer. This *in vitro* model is potentially useful for searching of Bcl-2 modulating agents, which may help

reverse drug resistance conferred by Bcl-2 overexpression.

四、計劃成果自評:

This study has been successfully completed. The results will be submitted for publication in the peer-reviewed journal as soon as possible.

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