行政院國家科學委員會專題研究計畫　成果報告

鼻咽癌之抗藥性及其逆轉：著重於 EB 病毒、一般抗藥機制、及 HER 訊號傳遞徑路之研究(3/3)

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□赴國外出差或研習心得報告一份

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

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行政院國家科學委員會專題研究計畫成果報告

計畫名稱：鼻咽癌之抗藥性及其逆轉—著重於 EB 病毒、一般抗藥機制、及 HER 訊號傳遞徑路之研究(3/3)

Drug Resistance and Its Reversal in Nasopharyngeal Carcinoma--A systemic study on the role of EB virus, Classical drug resistance mechanisms, and signal transduction pathway of human epidermal growth factor receptors (HER)

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一、中英文摘要

中文摘要

鼻咽癌是國人最重要的惡性腫瘤之一。由於治療學上的進步，現今 50-90% 的局部鼻咽癌患者已可被治癒；然而大多數復發或轉移的鼻咽癌患者，預後仍十分不好。對此類病患而言，化學治療雖然在開始治療時極為有效，但卻經常因為癌細胞對化學治療藥物產生抗藥性而失敗。過去有關鼻咽癌的抗藥性機轉的研究並不完整，我們希望藉由本研究能針對此一問題做一系統性的探討。

在此一三年計劃中，我們嘗試以三個研究面向來探索這個問題：(1). 傳統抗藥機轉在鼻咽癌細胞化學治療抗藥性所扮演的角色；(2). HER family 在鼻咽癌細胞化學治療抗藥性所扮演的角色；(3). EB 病毒在鼻咽癌細胞化學治療抗藥性所扮演的角色。研究方法包括有體外細胞株研究，及臨床病理相關性研究。

首先，我們以臨床病理相關性研究方法來探討傳統的抗藥機轉。我們從 202 位鼻咽癌患者中挑選了 44 位，這 44 位患者均因為復發或轉移之鼻咽癌而接受化學治療為唯一治療策略，而且他們治療前之組織均也完整地保留。MDR1、GSTπ及 p53 以免疫組織染色測定，進一步與病患化學治療的成績（包括對化學治療的反應率及病人的存活期）做相關性研究。我們的結果顯示：MDR1 是預測病患存活的不好因子，與化學治療之反應率並無相關。P53 之高表現卻與較高的化學治療敏感度呈正相關；P53 高表現病患的存活也有較好之趨勢。

至於 HER family 之調控與鼻咽癌細胞株的化療藥物敏感度的研究方面： 首先我們確定 NPC-TW01, NPC-TW04 及 HONE1 三株鼻咽癌細胞株均表現 HER1 (EGFR) 的 mRNA 及蛋白質；然而，三株鼻咽癌細胞株 HER1 的表現量均少於 A431（A431 是一株 HER1 表現量十分高的上皮細胞癌）。使用不同的抑制 HER1 的藥劑, 如 PD153035 或 ZD1839 等 HER1 的 tyrosine kinase 抑制劑、單株抗體 mAb225 等，均可造成鼻咽癌細胞株生長的抑制；惟其抑制細胞生長的 IC_{50} 均比 A431 的 IC_{50} 來得高。我們進一步針對 NPC-TW04 細胞進行合併 HER1 抑制劑及各種抗癌藥物的實驗。再同時投予 HER1 抑制劑及各種抗癌藥物時，藥物的合併效果僅僅呈相互拮抗~相加之效果；但若在「經 PD153035 或 ZD1839 處理 24

小時後再加抗癌藥物」則有較好的 synergistic 效果。此外，我們也進一步發現使用 PI-3K 抑制劑（LY294002，可抑制 PI-3K/Akt 之訊息傳遞徑路）與各種抗癌藥物同時使用，可以達到十分明顯的 synergistic 效果。

有關 EB 病毒與鼻咽癌細胞化學治療抗藥性的研究方面，我們先確認了三株鼻咽癌細胞株均不表現 EB 病毒的各種基因後，再以基因轉殖方式將 EB 病毒的 Zta 蛋白轉殖入 NPC-TW04 細胞株，經篩選並確認有四株轉殖細胞株，這四株轉殖細胞株均表現 Zta，而且這些轉殖細胞株對不同的化療藥物的毒殺作用均較其原始細胞更不敏感。這些資料顯示：在鼻咽癌細胞株大量表現 Zta 會導致癌細胞抗藥性的產生，其可能的分子機轉正進一步深入探討中。

關鍵詞：鼻咽癌，化學治療抗藥性，EB 病毒，EB 病毒之 Zta 蛋白，Human epidermoid growth factor receptor (HER)

Abstract

Nasopharyngeal carcinoma (NPC) is one of the major endemic cancers in Taiwan. Although 50 to 90% of loco-regional NPC patients can be cured by definitive local treatments, the outcome of patients with recurrent or metastatic NPC remains poor. The successful use of systemic chemotherapy, despite high tumor response rate initially, is limited by the emergence of drug resistance in cancer cells. The mechanisms of drug resistance to chemotherapy in NPC have been largely unknown. We thus proposed this 3-year project to study this important issue systemically. Three possible drug resistance mechanisms have been covered, including classical drug resistance mechanisms, the human epidermal growth factor receptor (HER) family, and EBV-viral proteins.

Regarding the classical drug resistance mechanisms of NPC, we performed a clinico-pathological correlation study. From 202 archive NPC patients, 44 who received systemic chemotherapy for recurrent or metastatic diseases were included because of complete medical records and adequate pre-treatment tissue specimens. Immunohistochemical staining of the expression of MDR1, GSTπ, and p53 were correlated with the treatment outcomes of the patients. The results indicate that MDR1, although correlated with a poor overall survival, did not appear to predict chemoresistance of NPC. Over-expression of p53 by immunohistochemical staining, was associated with a better response rate to systemic chemotherapy and a trend towards better survival.

As for the study of modulation of human epidermoid growth factor receptors in relation to chemoresistance in NPC, 3 NPC cell lines, including NPC-TW01, NPC-TW04, and HONE-1, were employed for *in vitro* studies. All 3 NPC cell lines expressed mRNA and protein of EGFR, although with expression levels lower than that of A431, an EGFR-overexpressing cell line. Two EGFR-specific tyrosine kinase inhibitors (PD153035 or ZD1839) and a monoclonal antibody to EGFR (mAb 225) inhibited the growth of NPC cells with IC_{50}s higher than those for A431 cells. The combination effect of EGFR inhibitors and cytotoxic agents were further explored in NPC-TW04 cells. Concomitant treatment of EGFR-inhibitors and cytotoxic chemotherapeutic agents did not improve the growth inhibitory effect. With the pretreatment of PD153035 or ZD1839 for 24 hours followed by cytotoxic agents, the cytotoxicity of several anticancer drugs, including doxorubicin, paclitaxel, cisplatin, and 5-fluororuacil, was improved with the combination index well below 0.5 at fraction inhibition >70%. Further, when cytotoxic chemotherapeutic agents were combined with LY294002, an inhibitor of PI-3K/Akt pathway which was downstream to EGFR, a significantly better synergism was demonstrated. The data suggest that combination of EGFR inhibition with cytotoxic drugs may be further developed as a treatment modality for NPC.

Finally, we also tried to evaluate the possible role of Epstein-Barr virus (EBV) in the development of drug resistance in NPC cells. We first characterized that there was no expression of several EBV viral genes, including BamH1W, BZLF1, and EBNA1, in all 3 NPC cell lines. Zta (ZEBRA, Z, encoded by BZLF1 of EBV), a transcriptional factor responsible for the switch of viral latency into lytic cycles, was selected because several previous studies have shown a close interaction between this viral proteins and several host proteins, including p53, NF-κB, and cyclin kinase inhibitors, which have all bee linked to drug resistance in human cancer cells. At least 4 stable transfectants of a Zta-expressing vector, pRCMV-Zta, were selected from NPC-TW04. Overexpression of Zta message was confirmed by real-time RT-PCR. The IC_{50}s to different kinds of cytotoxic agents in these transfectants were much higher than those of parental lines and vector-control lines, especially to paclitaxel and doxorubicin. These data suggest that over-expression of Zta in NPC cells confers a phenotype of resistance to multiple cytotoxic drugs *in vitro*. The possible mechanisms underlying this phenotype are now under investigation.

Keywords ： Nasopharyngeal carcinoma (NPC), Chemoresistance, Epstein-Barr virus (EBV), Zta protein of EBV, Human epidermoid growth factor receptor (HER)

二、 計畫緣由與目的 (Background)

Nasopharyngeal carcinoma (NPC), well known for its distinctive epidemiologic features and association with Epstein-Barr virus (EBV), [1,2,3] is an important endemic malignancy of Taiwan. In 1998, NPC was responsible for more than 800 deaths in Taiwan. [4]

Radiotherapy is the standard treatment for loco-regional NPC. Recent progress on incorporating systemic chemotherapy into standard radiotherapy has improved the 5-year survival rates for patient with loco-regional NPC from 52 to 97%, depending on the clinical stages. [5-7] On the other hand, although combination chemotherapy may result in a high response rate in patients with recurrent or metastatic NPC initially, only less than 10% of these patients can survive more than 2 years. [1,3,8]

Inherent or acquired resistance to anticancer drugs is a major obstacle to the success of chemotherapy.[9] However, the mechanism of drug resistance of NPC have seldom been studied before.

Traditionally, the drug resistance mechanisms included those specific to drug exporters (such as MDR1 and MRP), detoxification mechanisms (such as GSTπ), and the altered drug targets. Recently, mechanisms related to cell death control and cellular stress response have also been linked to the resistance to chemotherapy. The roles of these markers in the prediction of chemosensitivity in NPC are largely unknown.

The expression of human epidermal growth factor receptor (HER) family, including EGFR (HER1), HER2, has been demonstrated in NPC tissues.[10-12] The expression of these markers correlates with advanced stage of disease, predicting a poor clinical outcome. [10] Recently, enhancing chemosensitivity by anti-HER1 or anti-HER2 strategies have been shown in both pre-clinical and clinical studies of a variety of human cancers. [13-15] Whether modulation of HER family can be employed as a treatment modality for NPC has not yet been addressed.

Further, we have been working on drug resistance mechanisms in several virus-associated malignancies for years. We have shown before that a large portion of EBV-associated T-cell lymphoma and Hepatitis B virus (HBV) related hepatocellular carcinoma (HCC), and HTLV-1 associated adult T-cell lymphoma and leukemia (ATLL) express drug resistance markers. [16-20] We also demonstrated that both tax protein of HTLV-1 and pX protein of HBV

transactivated *mdr-1* gene expression in T-cell lymphoma and hepatoma cell-lines, respectively.[21,22] Whether Epstein-Barr virus (EBV), which is closely associated with NPC and encodes several biologically active viral proteins [23,24] may also contribute to drug resistance of NPC is a testable hypothesis.

In order to understand the possible drug resistance mechanisms of NPC, we planned to investigate the 3 above-mentioned research categories, using different approaches.

Approaches (Materials and Methods):

Two approaches were adopted: (1) *in vitro* cell-line study; (2). clinico-pathological correlation study.

(1). *In vitro* cell-line study: Three NPC cell lines, including NPC-TW01, NPC-TW04, and HONE1, were maintained in our laboratory. First, these cells were characterized for the expression of EBV-encoded genes (EBNA1, BamH1W, BZLF1), classical drug resistance markers (MDR1, MRP, GSTπ, TS, TP), and HER family (HER1, HER2, HER3, HER4) by Western blotting and reverse transcriptase polymerase chain reaction (RT-PCR). The sensitivity of these cells to different anticancer drugs, such as cisplatin, 5-FU, doxorubicin, paclitaxel, were determined by MTT assay. Further, we used both negative modulation and positive modulation approaches to try to modify the drug sensitivity profiles of these cells. Specific inhibitors of known signaling pathways, blocking antibodies, and anti-sense approaches were part of negative modulation strategies; gene transfer by transfection or infection was used in positive modulation experiments.

(2). Clinico-pathological correlation study: NPC patients with advanced NPC received systemic chemotherapy as the sole mode of therapy for the recurrent or metastatic disease were included for the clinicopathologic correlation study provided their pre-treatment tissues were adequate for analysis. The tissues were sectioned and stained with proper antibodies by routine procedures. The clinical endpoints for the analysis were the response to chemotherapy and patients' survival.

三、 結果與討論

Results

Base-line Characters of NPC Cell-lines

The IC_{50}s to 4 different anticancer drugs for NPC-TW01, NPC-TW04, and HONE1 cells was tabulated in **Table1**. The classical drug resistance markers, including multi-drug resistance 1 (MDR1) and multidrug resistance- related protein (MRP) for doxorubicin and paclitaxel, glutathione-*s*-transferase π (GSTπ) for cisplatin, evaluated by RT-PCR; glutathione level measured by colorimetric assay for glutathione; and 4 members of HER family detected by RT-PCR and Western blotting were summarized in **Table 2**. The IC_{50} profiles in 3 cells did not differ very much, nor did the expression of the checked drug resistance markers. HER1 (EGFR) was universally expressed in 3 NPC cells, with the levels lower than that of A431, a well-characterized HER1-over-expressing cell line.

As for the expression of several EBV-related genes, we found no amplification of Bam H1W region of EBV genome and EBNA1 form 3 NPC cells by PCR. RT-PCR of BZLF1 and BHRF1 found no amplicon, either.

Correlation of the Expression of Classical Drug Resistance Markers in NPC tissues and Treatment Results of Recurrent or Metastatic NPC :

In a cohort of 202 consecutive patients diagnosed at the Department of Pathology of National Taiwan University Hospital, 44 patients were selected for this analysis. The correlation study was performed on the expression of MDR1, GSTπ, and p53 with tumor response to chemotherapy and survival of the patients.

Thirty-four patients received cisplatin-based regimens, and 28 of them were enrolled in a prospective trial using a

doxorubicin-containing regimen. The overall response rate was 70%. Expression of MDR1 was seen in only 5 cases (11%) and was associated with a significantly worse overall survival (P= 0.028), yet did not appear to predict chemoresistance to the doxorubicin-containing regimen. Over-expression of p53 was seen in 22 patients (50%), and surprisingly, was correlated with chemoresponse (P= 0.026) and a trend towards better survival. GSTπ expression was demonstrated in 13 cases (30%) and was not correlated with chemoresistance to cisplatin-containing regimens and overall survival (**Table 3 and Fig.1**).

Modulation of HER1 in NPC Cells

Since the 3 NPC cells express HER1, we tested several HER1-inhibitory strategies in NPC cells, focusing on the effects of growth inhibition of single agents or combination with cytotoxic drugs.

Anti-HER1 was achieved by specific monoclonal antibody (mAb225) or by two specific tyrosine kinase inhibitors (PD153035 and ZD1839). A HER1-overexpression cell line, A431, and a HER1-low-expression cell line, MCF7, were included for all of the studies. The IC50s of 3 NPC cells to PD153035 and ZD1839 were 10~20 μM and 20~30 μM, respectively. The IC50 to mAb225 of NPC-TW04 cells was about 3μg/ml. The inhibitory potency of these HER1 inhibitors in NPC cells, reflected by the lower IC50s, was inferior to that in A431 cells, but much higher that that of MCF7 **(Fig. 2 & Fig. 3)**.

The combination effect of EGFR inhibitors and cytotoxic agents were further explored in NPC-TW04. When EGFR inhibitors and cytotoxic agents were used concomitantly for 72 hours, the interaction between drugs were mainly antagonistic by median effect analysis, indicating no chemosensitization effect by EGFR inhibitors **(Fig.4** A~D). However, with pretreatment of HER1 inhibitors for 24 hours followed by cytotoxic agents, a synergistic interaction of either PD1530305 or ZD1839 with several anticancer drugs, including doxorubicin, paclitaxel, cisplatin, and 5-fluororuacil, can be demonstrated with combination index well below 0.5 at fraction inhibition >70% (**Fig.4** E-H).

Since PI-3K/Akt pathway, one of the important signaling pathways downstream to EGFR, have been shown to involve in resistance to chemotherapeutic agents in cancer cells. We further tested the effect of combining inhibiting PI-3K by LY294002 and cytotoxic chemotherapeutic agents in NPC-TW04 cells. Despite concomitant use of LY294002 and cytotoxic agents, a strong synergistic interaction was clearly demonstrated (**Fig. 5**).

EBV-encoded Zta Protein and Drug resistance of NPC Cells

The expression of BZLF1 transcript and its coded ZEBRA (Zta, Z, EB1) protein, the pivotal protein responsible for the switch of latent viral infection and lytic infection of EBV, has been demonstrated in NPC tumor cells. [25-27] Zta is a transcriptional factor and acts to activate immediate early and early genes of EBV lytic cycle. It has been reported before that Zta overexpression in human cells can transactivate several human genes. [28-31]

A Zta-constitutively expression vector (pRCMV-Zta) driven by CMV-promoter, was constructed, and was introduced to NPC-TW04 cells. At least 4 stable transfectants of Zta were selected from single cell cloning. Overexpression of Zta message was confirmed by real-time RT-PCR.

Cytotoxicity to various kinds of cytotoxic agents was compared in these 4 transfectants, vector-transfected cell line, and parental NPC-TW04 cells. The IC50s to different kinds of cytotoxic agents in these transfectants were much higher than those of parental lines and vector-control lines. **(Fig 4)**.

In order to exclude the possibility of effects conferred through the process of selection, we also checked the growth-inhibition in NPC-TW04 cells in non-selected Zta-overexpressing batches. The data was consistent with what we have observed in stably selected clones.

Discussion

The purpose of this study is to systemically explore the possible drug resistance mechanisms of NPC. Three major categories were covered, including classical drug resistance markers, such as MDR1, GSTπ, and p53, members of HER family, and EBV-encoded Zta protein.

In the clinico-pathologic study, we demonstrated that the expression of MDR1 is uncommon in NPC tissues, does not predict chemoresistance, and appears to be correlated with poor survival. Overexpression of p53 is found in half of the NPC patients and is correlated with a better treatment outcome.

While the significance of MDR1 has been shown to predict poor chemosensitivity and prognosis in many cancers,[32-38] the role of MDR1 in NPC has never been reported. On the contrary, there are some other tumors for which MDR1 expression is not correlated with chemoresistance.[39-42] Previous studies also pointed out that MDR1 is a marker of aggressive tumor behavior and a poor prognostic factor for patients' survival.[43] Our findings on NPC supported this notion.

Most previous reports indicated that altered p53 function is associated with poor prognosis of patients in a variety of human cancers.[44,45] Mutated p53 protein has longer half life and thus results in p53 overexpression in immunohistochemical examination. P53 over- expression has been identified as a poor prognostic factor in a variety of human cancers, including breast cancer, ovarian cancer, stomach cancer, lung cancer, and sarcomas. In some of these cancers, over-expressed p53 was further correlated with chemoresistance.[46] These data are in general consistent with the notion that p53 mutation results in a defective cellular apoptotic response to cytotoxic insults, and leads to chemoresistance of the tumors and a poor outcome. However, p53 over- expression, as detected by immunohistochemical examination, may not always indicate an underlying p53 gene mutation. For example, while p53 overexpression is detected in the vast majority of testicular cancers, p53 mutation is an extremely rare event.[47,48] It appears that a similar situation may exist in NPC, since p53 mutation is also a rare event in NPC, with a prevalence rate ranging from 0 to 20%.[49-53] In other words, the overexpression of p53 in NPC or testicular cancers may actually indicate more abundant functional wild-type or mutant p53 molecules, which contribute to a more effective p53-mediated repairing or apoptotic mechanisms following DNA damage. This may help explain the association between chemo-responsiveness and p53 over-expression in our NPC patients.

GSTπ is the prototype of molecules responsible for cellular resistance to cisplatin. Our data did not support its role in the clinical drug resistance of NPC. However, because of limited number of patients in this analysis, further larger-series studies are needed.

Regarding the modulation of HER1 (EGFR) in NPC, our findings showed that several anti-HER1 strategies inhibited the growth of NPC cells, and possibly have some impact on enhancing chemotherapeutic effect of multiple cytotoxic drugs in NPC cells. The results are basically compatible with previous reports on heed and neck squamous cell carcinoma (HNSCC).[10-12,54-56] Interestingly, our *in vitro* data did not support concomitant use of EGFR-inhibitor and chemotherapeutic agents, which showed basically antagonistic interaction in NPC cells. In stead, we demonstrate two possible ways to achieve chemosensitization effect by manipulating EGFR pathways. One is using the EGFR-inhibitor pretreatment sequence; the other possibility is to modulate molecules downstream to EGFR[57-59], which might be more relevant to be resistant to chemotherapy in cancer cells. Our preliminary result did support the PI-3K/Akt pathway is indeed worth of exploring in this angle.

Previous studies of anti-Zta antibody titer on NPC patients and immunohistochemistry of Zta on NPC tissues

supported the expression of Zta in the progressive and metastatic stages of NPC. [60] Overexpression of Zta in various types of cells resulted in alteration of cell cycle propagation, increased metastatic potential, and etc.. P53, cyclin-dependent kinase inhibitors, NF-κB, and tyrosine kinases have been found as targets of Zta overexpression. [28-31, 61-63] Our data did show Zta protein confer a drug resistant phenotype in NPC-TW04 cells. We currently are focusing on p53 and NF-κB, both of which have been linked to chemoresistance in a variety of cancer cells and have also been shown to have protein-protein interaction with Zta.

四、 計畫成果自評(Self Assessment)

In this 3-year study, we have made some progress on the planned 3 angles of drug resistance mechanism research of NPC cells. Part of our findings has been published[64]; others have been presented to annual meetings of ASCO 2002, USA, and annual meeting of AACR 2003. [65,66]

Our data have both clinical implication and basic research interest. For the former, our results not only reveal possible predictors for chemotherapy in NPC patients (MDR and p53), but also direct possible approaches to improve efficacy of cancer chemotherapy by modulating EGFR pathway on NPC cells. This information will be very useful in designing clinical protocols in the treatment of advanced NPC patients in the future. On the other hand, how the modulation of signaling pathways leads to chemosensitization effect remains to be clarified. Although our preliminary data indicate the importance of PI-3K/Akt, this surely needs further confirmatory studies. Finally, over-expression of a viral transcriptional factor, Zta, of EBV confers a multi-drug resistance phenotype in NPC cells. Further in-depth mechanistic studies may reveal interesting aspects in terms of the evolution of virus-host cell interaction and possible new approaches to reverse drug resistance in virus-associated malignancies.

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Table 1. IC_{50}s to Anticancer Drugs NPC Cell Lines

	NPC-TW01	NPC-TW04	HONE1
CDDP (μM)	5.3 ± 2.5	3.0 ± 0.1	1.8 ± 0.2
5-FU (μM)	11.4 ± 5.2	8.4 ± 3.9	17.3 ± 3.2
DOXO (nM)	0.27 ± 0.02	0.44 ± 0.06	0.27 ± 0.05
Taxol (nM)	3.0 ± 4.7	59.3 ± 102	6.4 ± 5.4

Table 2. Drug-resistance Markers in NPC cells

	NPC-TW01	NPC-TW04	HONE1
MDR1[1]	--	--	--
MRP[1]	+	+	+
GSTπ[1]	+	+	+
G-SH [3]	21.85	17.76	16.95
HER1[2]	+	+	+
HER2[2]	+*	+*	+*
HER3[1]	--	--	--
HER4[1]	--	--	--

1. Checked by RT-PCR; 2. Checked both by RT-PCR and Western blotting; 3. Checked by colorimetirc glutathione detection kit (expressed as μM/μg of cellular protein)

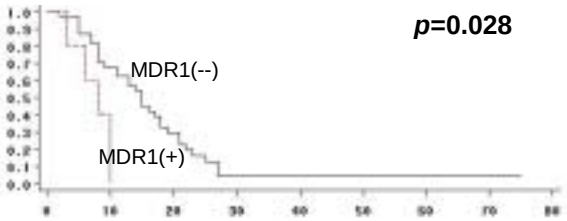
* The expression of HER2 in under verification.

Table 3. Correlation of MDR1, GSTπ, p53 with Treatment Response for 44 NPC patients

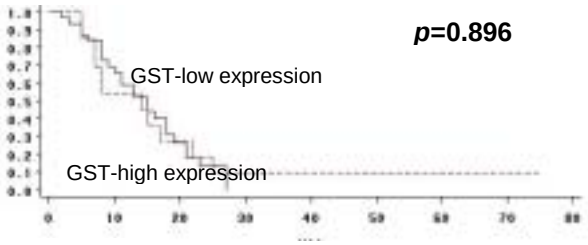
		Response rate	*P*-Value
MDR1	--	72	
	+	60	0.589
GSTπ	--	74	
	+	62	0.404
P53 Over-expression	--	55	
	+	68	0.026*
MDR1 (Doxo-+ C/T)	--	79	
	+	75	0.819
GSTπ (CDDP-+ C/T)	--	80	
	+	67	0.419

Fig.1 Survival curves for patients with metastatic or recurrent NPC treated with systemic chemotherapy. Analysis was mode according to the expression of different markers: (A), MDR1; (B) GSTπ; (C)p53.

(A)



(B)



(C)

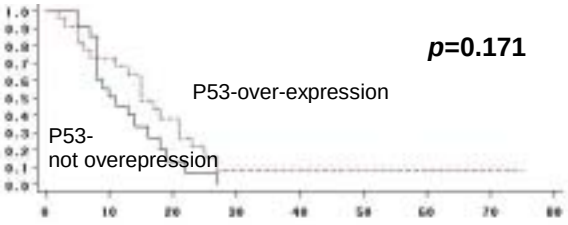


Fig 2. Growth Inhibitory Effect of HER1-specific tyrosine kinase inhibitors (PD153035, left; ZD1839, right) in NPC cells, A431 cells, and MCF7 cells.

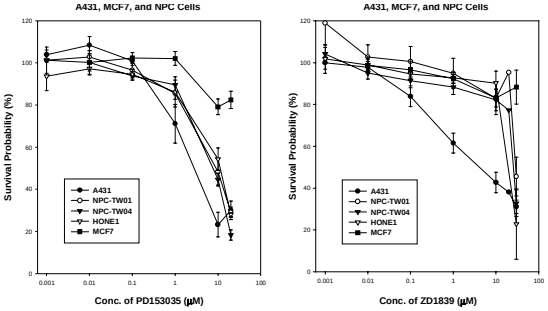


Fig 3. Growth Inhibitory Effect of mAb225 (a murine monoclonal antibody against EGFR) in A431 and NPC-TW04 cells. cells, and MCF7 cells.

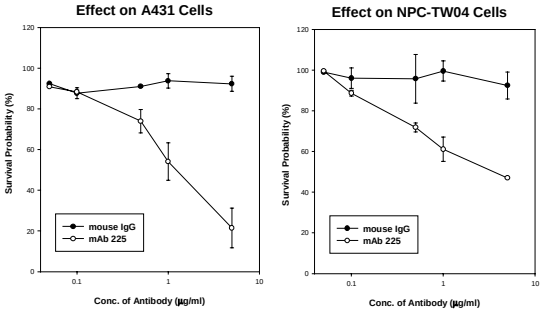


Fig 4. Combination effect of EGFR-TK inhibitors (PD98059 or ZD1839) and different cytotoxic chemotherapeutic agents in growth-inhibitory effect of NPC-TW04 cells, evlauted by median effect analsyis.

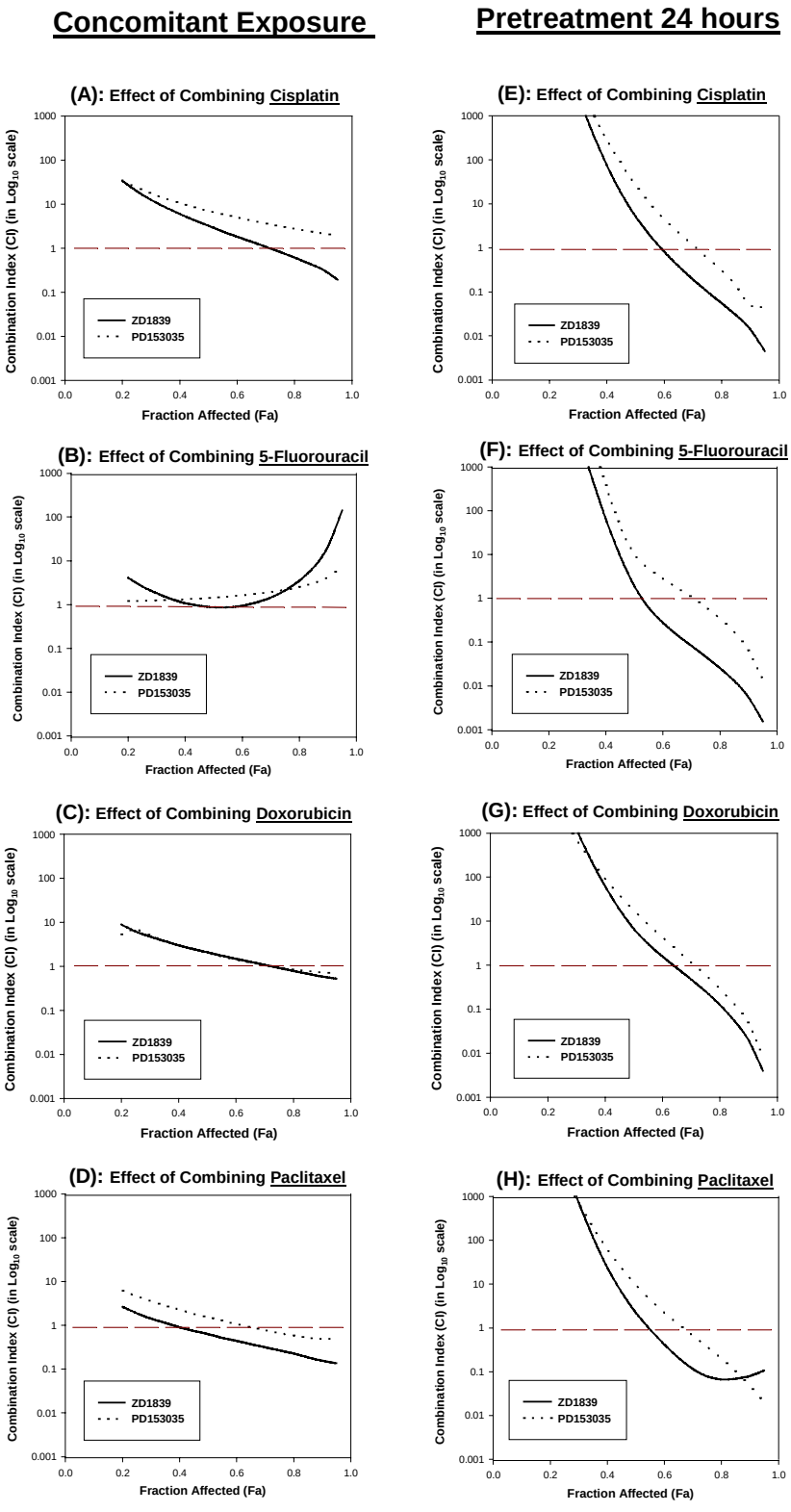
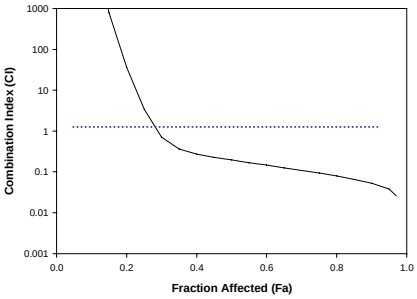
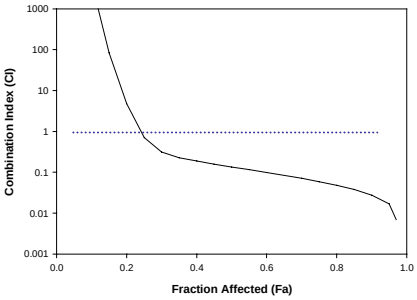


Fig 5. Combination effect of PI-3K/Akt inhibitor (LY294002) and different cytotoxic chemotherapeutic agents in growth-inhibitory effect of NPC-TW04 cells: Cells were treated with both drugs concomitantly for 72 hours.

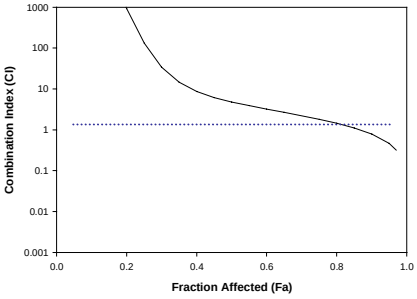
(A) Doxorubicin



(B) Paclitaxel



(C) Cisplatin



(D) 5-Fluorouracil

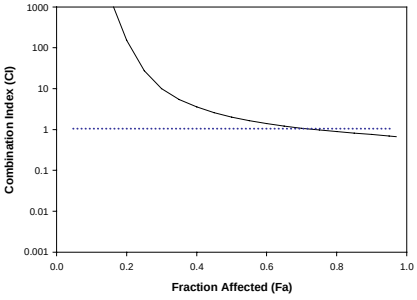
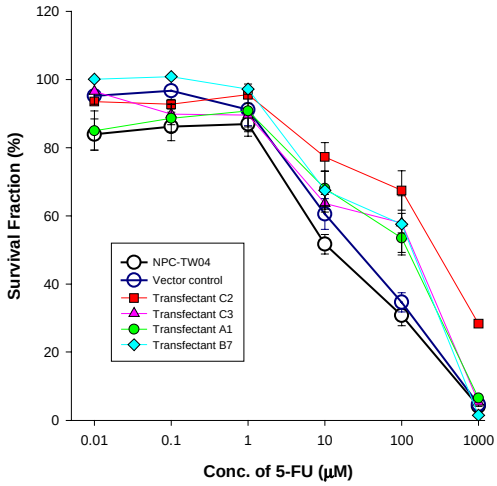


Fig 6 Cytotoxicity Effect of 5-FU (panel A) and Doxorubicin (panel B) in NPC-TW04 cells, vector control cells, and pRCMV-Zta- over- expressing transfectants (C2,C3, B7, A1). (Data regarding cisplatin and paclitaxel, which showed similar tendency was not included in this figure.)

(A)



(B)