行政院國家科學委員會專題研究計畫　期中進度報告

鼻咽癌的抗藥機轉研究：抗藥基因、EB 病毒基因、化學激素、化學激素受體在腫瘤細胞的表現和相關性(1/3)

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共同主持人：鄭安理
計畫參與人員：林亮音

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附件一

行政院國家科學委員會補助專題研究計畫 □ 成 果 報 告 ■期中進度報告

鼻咽癌的抗藥機轉研究：抗藥基因、EB 病毒基因、化學激素、化學激素受體在腫瘤細胞的表現和相關性

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共同主持人：鄭安理
計畫參與人員：林亮音

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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執行單位：台北醫學大學病理學科

中　華　民　國　93　年　5　月　30　日

中文摘要

關鍵詞：鼻咽癌、化學治療抗藥性、EB 病毒基因

鼻咽癌是國人常見的頭頸部惡性腫瘤之一。對原發腫瘤作放射線是治療鼻咽癌最常用方式，但因多數鼻咽癌病例的臨床分期為第 III 期以上，因此常在放射線治療後有腫瘤復發或遠端轉移，這類病人的預後仍十分不好。對此類病患，化學藥物是治療的另一選擇；有時對末期鼻咽癌病患，合併放射線和化學治療是唯一的治療方法。化學藥物對原發、再發或轉移的鼻咽癌雖然在治療初期極為有效，但卻經常因腫瘤對化學治療藥物產生抗藥性而逐漸失敗。我們以切片的原發、再發和轉移鼻咽癌腫瘤組織，作分子和免疫組織化學染色研究，希望能對鼻咽癌腫瘤細胞產生抗藥性的各種可能機轉做更深入的探討。

在過去的研究中，我們曾觀察到 EB 病毒有關之 T 細胞淋巴瘤有多重抗藥基因表現增加的現象，顯示 EB 病毒的存在可能與腫瘤的抗藥性有關。EB 病毒與鼻咽癌的密切相關性是眾所周知，某些 EB 病毒蛋白已知會影響細胞對外來壓力的反應，改變細胞週期進行，並影響細胞凋亡的現象。而這些 EB 病毒蛋白是否會影響鼻咽癌細胞對化學治療藥物的反應，則有待進一步研究。

抗藥機轉，如 Multidrug Resistance-1 (MDR-1)，Glutathione-S-transferase-π (GST-π)，thymidylate synthase (TS)，p53 及 bcl family 等在鼻咽癌腫抗藥性的角色，到目前為止，相關資料仍十分有限，我們過去的研究顯示：MDR-1 的表現會使鼻咽癌預後變差，p53 的表現卻預測好的治療效果。但是鼻咽癌在化學治療後產生抗藥性的機轉，仍需更進一步探討。

過去研究已証實有一部份的鼻咽癌組織表現 HER (Human epidermal growth factor receptor) family，如 EGFR (HER-1)、HER-2 (neu)等。這些標記的表現通常與疾病的嚴重度相關，也是預後不好的指標。近年來，對抗 HER-2 及對抗 EGFR 的藥物，在實驗室及臨床研究中均証實可以加強化學治療的效果。然而，此一治療策略尚未在鼻咽癌中嘗試。

本研究是一個為期三年的連續性研究計畫，本年進行的研究內容主要將達成的目標為二部分：

1.以免疫組織化學染色法進行對約 150 例原發、再發和轉移性鼻咽癌腫瘤作各種鼻咽癌腫瘤特殊基因（如 p53、bcl-2、bax、bcl-X、EGFR）、EB 病毒之潛伏膜蛋白（Latent membrane protein-1，LMP-1）、抗藥基因（如 MDR-1、GST-π、TS）的表現情形。

到目前本研究已完成 151 例原發、再發和轉移性鼻咽癌腫瘤免疫組織化學染色的部分。得到的結果發現 151 例原發性鼻咽癌中有 40.4%表現 EB 病毒 LMP-1，再發性者陽性率為 38.6%，轉移性病例則為 45%。p53 在多數鼻咽癌，包括原發性（86.1%），再發性（84.1%）和轉移性（95%），都會表現。Bcl-2 的表現更多，分別為 90%、86.7%和 100%。Bax 和 bcl-x 的表現也很強，分別為 94.7%、100%和 91%，與 91%、100%和 100%。鼻咽癌的抗藥基因表現方面，本研究發現 MDR-1 在多數鼻咽癌並不表現（86.8%、75%和 100%），GST-π則在約 60%的鼻咽癌會表現（57.6%、68.2%和 65%）。

2.以雷射細胞摘取技術（Laser Capture Microdissection, LCM）摘取冷凍組織切片中的腫瘤細胞，進行 MDR-1，GST-π，TS，p53，bcl-2，bax，bcl-$X/_L$，bcl-$X/_S$,，EB 病毒 LMP1 基因，EGFR，HER-2 或其他 HER family 等基因的表現情形。此部分本年度預計進行原發、再發和轉移鼻咽癌各約 5 例，目前本部分研究尚未得到合適的成果但正加緊進行中。

我們相信本研究持續進行，則鼻咽癌腫瘤的抗藥機轉、EB 病毒基因、HER family 在鼻咽癌抗藥性之角色可以獲得釐清，藉由此臨床與病理相關性研究，可以找出最具有臨床治療意義的生物指標，有助於進一步提昇轉移或復發鼻咽癌病患接受化學治療的治療成績。

英文摘要

Keywords：Nasopharyngeal carcinoma (NPC), Chemoresistance, Epstein-Barr virus (EBV)

Nasopharyngeal carcinoma (NPC) is an important endemic cancers in Taiwan. Although radiotherapy is the treatment of choice for loco-regional NPC but for recurrent or distant metastasis systemic chemotherapy is the only choice for those patients and is usually effective initially but limited by the emergence of drug resistance of cancer cells. However, the mechanisms underlying the drug resistance in NPC has never been adequately studied.

Previously, we observed that a large portion of EBV-associated T-cell lymphoma express drug resistance markers. We hypothesized that virus-encoded viral proteins contribute to clinical drug resistance. Epstein-Barr virus (EBV), which is closely associated with NPC, encodes several biologically active viral proteins have been known interaction with cellular stress responses, cell cycle propagation, and apoptosis. It is important to clarify if these EBV-encoded viral proteins may contribute to drug resistance of NPC. It is also important to clarify the role of drug resistance markers, e.g. MDR-1, GST-π, TS, p53, and bcl family, in NPC.

The expression of human epidermal growth factor receptor (HER) family, including EGFR (HER-1) and HER-2, has been demonstrated in NPC tissues. The expression of these markers correlates with advanced stage of disease, predicting a poor clinical outcome. Recently, enhancing chemosensitivity by anti-HER-2 or anti-EGFR strategies have been shown in both pre-clinical and clinical studies. The possibility of improving treatment result of NPC by modulating HER family has not yet been addressed.

In this study, we have performed the following works in the first year:

1. Detection of the expression of the apoptosis-related proteins (p53, bcl-2, bax, and bcl-X), drug resistance proteins (MDR-1, GST-π, and TS), and EBV proteins, the LMP1, and EGFR in tumor tissues of primary, recurrent, and metastatic NPC by immunohistochemical method using specific antibody. The antibodies used are all commercially available.

   One hundred fifty-one primary, recurrent, and metastatic NPC were included in this part. The results showed that LMP-1 was expressed in 40.4% of primary NPC, 38.6% of recurrent NPC, and 45% of metastatic NPC, respectively. Wild type p53 protein was expressed in most NPC specimens, the results were 86.1% in primary, 84.1% in recurrent, and 95% in metastatic NPC. The bcl-2, bax, and bcl-X proteins were consistently expressed in NPC. About expression of drug resistance gene in NPC, low expression of MDR-1 and TS but highly expression of GST-π was found in primary, recurrent, and metastatic NPC.

2. Detection of mRNA of MDR-1, GST-π, TS, p53, bcl-2, bax, bcl-X, the EBV LMP1, HER-1 (EGFR), and HER-2 (neu) genes in tumor cell by Laser Capture Microdissection (LCM) for dissecting tumor cells from frozen sections of NPC biopsies. In first year, these studies will be finished in 5 each cases of primary, recurrent, and metastatic NPC. Unfortunately, these studies are still undergoing and no available data can be presented now.

According the above immunohistochemical results, we had great achievement in exploring the role of apoptosis-related proteins, classical drug resistance markers, EBV-viral proteins, and HER family in drug resistance of NPC. These information will be invaluable for further improving of the results of systemic chemotherapy for patients with advanced and metastatic NPC.

報告內容

Introduction

Nasopharyngeal carcinoma (NPC) is an important endemic malignancy of Taiwan and is strongly associated with Epstein-Barr virus (EBV). Radiotherapy is the standard treatment for loco-regional NPC and results in a 5-year survival rate around 40% in Taiwan. The survival is influenced by the initial clinical stage; and the recurrence is predicted by the bulky nodal disease. Recently, efforts have been devoted to incorporating systemic chemotherapy with radiotherapy to treat high-risk loco-regional or metastatic diseases and have promising results.

For patients with recurrent or metastatic NPC, systemic chemotherapy is the most important modality of treatment although the results is still not good. Cisplatin, cyclophosphamide, doxorubicin, vincristine, 5-fluorouracil (5-FU), and methotrexate are the major agents for treating NPC. Either of these agents used singly or in combination, although with very high response rate in advanced NPC patients, consistently results in low 2-year survival rate. Further NPC patients with recurrent or metastatic diseases respond particularly poor to chemotherapy as compared to chemo-naive loco-regional disease. In other words, with progression or metastases of the primary disease, NPC cells rapidly develop resistance to anti-cancer drugs. The mechanism underlying this phenomenon remains obscure. However, significantly increased number of EBV particles has been demonstrated in the metastatic NPC cells, and thus raises the possibility that virus may have played a role in this scenario. In this study, we plan to explore the possible role of EBV-encoded viral genes and proteins, several drug resistance markers and HER family in the drug resistance of NPC tumor tissues.

Aim

In this three-year-based research project, we will perform the following studies in first year to verify the possible mechanisms of drug resistance in NPC tumor tissue:

1. Detection of expression of different proteins in tumor cells of primary, recurrent, and metastatic nasopharyngeal carcinoma using immunohistochemical method on formalin fixed paraffin-embedded sections. The studied proteins include p53, bcl-2, bax, bcl-X, EGFR, LMP-1 of EBV, MDR-1, GST-π, and TS. The tissue samples will include about 150 cases of primary, recurrent, and metastatic NPC specimens.
2. For studying the genes of MDR-1, GST-π, TS, p53, bcl-2, bax, bcl-$X/_L$, bcl-$X/_S$, the EBV LMP1, EGFR, HER-2, and other HER family in tumor cells of primary, recurrent, and metastatic NPC, we used Laser Capture Microdissection (LCM) method for dissecting tumor cells from frozen sections of NPC biopsies.

Literature Review

1. Roles of Viral Proteins in Drug Resistance of Cancers

The possible association of viruses and resistance to anti-cancer treatments was suggested by several clinical observations. In EBV-associated peripheral T-cell Lymphoma (PTCL), a clinicopathological study demonstrated that the presence of EBV in PTCL was associated with a more aggressive clinical course.[1] And, most importantly, the expression of MDR-1 and GST-π was significantly higher in EBV-positive PTCL.[2]

2. Implication of EBV in Progression and Dug Resistance of NPC

Several evidences indicate that EBV might play a role in the progression of NPC. When both primary and metastatic NPC tissues were examined, the expression of EBER1 was much higher in metastatic lesions than the primary sites, indicating higher amount of EBV in the metastatic lesions.[3] In addition, elevated titers of antibodies to ZEBRA and EA, both are lytic-cycle proteins of EBV, preceded the development of recurrent or metastatic disease.[4-5] These evidences strongly suggest that the activation and proliferation of EBV is closely associated with progression of NPC. LMP-1 was shown to affect cellular stress response, cell cycle control, and apoptosis regulation and was expressed in NPC tumor cells of various degrees. We hypothesize that LMP-1 might contribute to the clinical drug resistance of NPC. In particular, the very poor response to chemotherapy of the metastatic NPC may be partially explained.

LMP1: A multi-potential viral protein

Latent membrane protein 1 (LMP1) has been extensively studied in many EBV-related malignancies because of its multiple biologic activities. It transforms rodent fibroblasts, changes the phenotypes of B-lymphocytes and prevents them from apoptosis by up-regulation of bcl-2. In epithelial cells, introduction of LMP1 leads to the alteration of normal differentiation, expression of EGFR, and expression of several anti-apoptotic molecules.[6-8]

This protean biologic activity of LMP1 can be explained partly by its structural resemblance to TNF receptor family.[9-10] LMP1 acts as a constitutively activated receptor, interacting with downstream signaling pathways, including NF-κB.[8] Recently, several reports have demonstrated that NF-κB plays a critical role in cellular response to anti-cancer treatments. In general, suppression of NF-κB pathway promotes apoptotic reaction of the cells to chemotherapeutic drugs, irradiation, and TNF.[11-13]

3. Correlation of Expression of EBV Viral Proteins and Chemoresistance of NPC

There are only few articles discussing the correlation and importance of EBV-viral proteins on clinical drug resistance of NPC. The only one article, which evaluated the expression of LMP1 in NPC tissues and the treatment outcome of the patients, was hardly informative because of incompleteness of clinical data and very small sample size.[14] In the present proposal, we plan to re-address this question with a more systemic and sophisticated approach.

The Roles of Classical Drug Resistance Markers in NPC

Resistance to a broad spectrum of anti-cancer drugs is the major obstacle to effective treatment of cancer patients. Several classical molecular mechanisms have been described in the past two decades. These classical drug resistance mechanisms include: (1) to decrease the intracellular concentration of drugs by a drug pump; (2) to decrease the intracellular concentration of drugs by increasing drug metabolism; (3) to alter the drug target, interfering with the interaction of specific drug with its target.[15] On the other hand, the cytotoxic effect of anti-cancer drugs works through the process of programmed cell death after the binding of the drug to its target. These post-target-binding events may be important in the phenomenon of multi-drug resistance.[16-17]

1. Classical Drug Resistance Mechanisms of Conventional Anticancer Drugs:

The first well-characterized specific drug resistance marker is MDR-1. MDR-1 encodes p-glycoprotein which is an membrane pump for many natural product drugs, including doxorubicin, vinca alkaloids, etoposide, and paclitaxel.[18-19] The presence of increased expression of MDR-1 in several types of tumor has been correlated with poor clinical response rate of chemotherapy and poor survival.[20] The MDR-1 reversing strategies were tested in several clinical trials.

Glutathione, as a coenzyme with a variety of enzymes, including glutathione-S-transferase (GST), plays a central role in metabolism or detoxification of drugs.[21] It is demonstrated that the cross-talk between intracellular glutathione-mediated detoxification and platinum accumulation is important in cisplatin resistance.[22]

High expression of thymidylate synthase (TS), the target enzyme of 5-fluorouracil (5-FU), has been demonstrated to be associated with chemoresistance to 5-FU-based chemotherapy in colorectal cancers.[23-24] Our previous study on gastric cancers showed that the expression of TS, detected by immunohistochemistry, reliably predicted the poor response to 5-FU-based chemotherapy and short survival time of the patients.[25]

2. Apoptosis-Related Mechanisms in Drug Resistance of NPC:

Most anticancer drugs induce apoptosis as a mechanism of cytotoxicity.[26] Failure to induce apoptosis is being recognized as an important category of multi-drug resistance.[27-28] p53-dependent apoptosis is one of the major pathways.[28]

p53, a tumor suppressor gene, acts as a gatekeeper in cell cycles. It detects DNA damages and prevents the G1/S propagation, allowing either DNA repair or apoptosis.[29] An intact p53 molecule is thus a pre-requisite for the cancer cells to go to apoptosis in response to anti-cancer drugs.[30-31] Over-expression of p53 due to mutated p53 has been shown to confer chemoresistance in various cancers.[32-35]

bcl-2 is an oncogenic protein that acts by inhibiting programmed cell death.[36] The expression of bcl-2 confers resistance to a variety of DNA-damaging agents. The expanding members of bcl-2 family fall into two opposing classes depending whether they induce or repress apoptosis.[37-38] The ratio of pro-apoptotic bcl-2 homologues (such as bax) and anti-apoptotic homologue (such as bcl-2) may decide whether the cells go to apoptosis in response to anti-cancer drugs.

bax and bcl-X are also oncogenic proteins that involved in apoptosis. bax can promote apoptosis. The long transcript of bcl-X (bcl-$X/_L$) can protect cell from apoptosis while the short transcript (bcl-$X/_S$) has adverse effect. These two apoptosis-related genes have not been studied in NPC before.

Although the expression of p53 and bcl-2 in NPC was described before,[38-40] whether these markers can predict the treatment results of systemic chemotherapy has never been addressed.

Human epidermal growth factor receptor (HER) family is a family of receptor tyrosine kinase. There are four members in this family, including EGFR (HER-1) and HER-2/neu.[41-43] These molecules are integral membrane receptor proteins for growth factors. Upon the ligand binding to its receptors, receptor dimerization occurs, followed by autophophorylation, and subsequent signaling cascades.[41-42] Both EGFR and HER-2 were demonstrated to involve in cell

proliferation, cell cycle control, apoptosis, and DNA repair, etc.[43]

Several studies have shown that the expression of EGFR and HER-2 is highly prevalent in NPC. Both the wild-type LMP1 or mutant LMP1 have been demonstrated in epithelial cell lines to confer the activation of EGFR.[44-45] The expression of both receptors is correlated with the process of malignant transformation and advancement of disease status.[46-47] The role of these two growth factor receptors have been further elaborated regarding the role of autocrine growth in NPC cell culture. Inhibition of EGFR receptor pathway, either by antagonizing monoclonal antibody or by tyrosine kinase inhibitors, lead to growth inhibition of NPC cell culture and tumor formation in mice.[48-51] On the other hand, the expression of HER-2 was identified as a poor prognostic factor for NPC patients.[52]

Inherently high expression of HER-2 or over-expression of HER-2 conferred resistance to various anti-cancer drugs in different cell lines.[53-56] The blockage of HER-2 pathway, which can be achieved by monoclonal antibody against receptors, antisense approach, oligo-peptides interfering the receptor dimerization, or tyrosine-kinase inhibitors, can reverse the HER-2 over-expressed phenotype and theoretically would lead to a reversal of drug resistance.[57-59]

In light of these encouraging pre-clinical results, the era of monoclonal antibody against human cancers has been started. A humanized version of anti-HER-2 antibody has proven to be active in patients of breast cancer, with single agent activity of 11.3 to 23%.[60-62] Most interestingly, the addition of anti-HER-2 antibody enhances the efficacy of chemotherapy.[63-64] As shown in a large scale phase III randomized trial or metastatic breast cancer, patients who received anti-HER-2 antibody and chemotherapy had much higher (20-40% increase) tumor response rate and longer time of tumor control than those who received chemotherapy alone.[64]

In conclusion, the clinical data suggested that concomitant use of anti-HER-2 or anti-EGFR therapy may enhance the effect of systemic chemotherapy on HER-2 or EGFR-over-expressed tumors. NPC, with its high EGFR and HER-2 expression, is potentially a good candidate for this approach.[65]

Material and Methods

A. NPC Sample Collection:

Freshly frozen and paraffin-embedded primary, recurrent and metastatic NPC tissue samples were collected by ENT physicians and also pooled from the Department of Pathology, National Taiwan University Hospital (NTUH). The clinical treatment response, results, outcome, and survival of these patients will be collected for later analysis.

B. Studying the Role of Drug Resistance Markers, EBV-Encoded Viral Proteins and HER Family in Drug Resistance of NPC:

To explore the role of drug resistance markers, EBV-encoded genes and proteins, apoptosis-related proteins, and HER family in drug resistance of NPC, the following works were finished in the past year:

1. Immunohistochemistry

Formalin-fixed paraffin-embedded tumor tissue samples of primary NPC tumors from primary and recurrent NPC patients (n=132) and metastatic NPC tumors (n=19) were pooled and

collected. Histopathologic classification of primary and recurrent NPC samples was based on the revised histologic classification of tumors of upper respiratory tract and ear by the World Health Organization (WHO) in 1991.

For immunohistochemical studies, serial paraffin sections were used and cut in 6 um in thickness. The paraffin sections were depaffinized and rehydrated using descending alcohol. After antigen retrieval, these sections were incubated with adequate commercially available monoclonal or polyclonal antibodies followed by adequate linked antibody and colorized by diaminobenzidine (DAB) using a standard indirect avidin-biotin-peroxidase method. Then the sections were counterstained with Mayer's hematoxylin solution. The IHC results were arbitrarily classified into four scores dependent on the intensity of immunoreactivity: 0, negative immunostaining; 1+, <10% tumor cells with positive immunostaining; 2+, 10–50% tumor cells with positive immunostaining, and 3+, >50% tumor cells with positive immunostaining.

2. Detection of mRNA by Laser Capture Microdissection and Real-Time RT-PCR

Laser capture microdissection (LCM) method will be used for dissecting tumor cells from frozen sections and paraffin sections of NPC biopsies for studying the expression genes of MDR-1, GST-π, TS, p53, bcl-2, bax, bcl-X, the EBV LMP1, and EGFR, HER-2 and other HER family in tumor cells. In first year, these studies will be done in 5 each cases of primary, recurrent, and metastatic NPC.

Results and Discussion

A. Immunohistochemistry

1. Detection of the expression of different gene products in tumor tissues of primary, recurrent, and metastatic NPC by immunohistochemical method using specific antibody. The studied proteins include apoptosis-related proteins (p53, bcl-2, bax, and bcl-X), drug resistance proteins (MDR-1, GST-π, and TS), and EBV proteins, the LMP1, and EGFR. The antibodies used are all commercially available.

So far, we finished the detection of the studied proteins in about 150 cases of primary, recurrent, and metastatic NPC. The results showed that LMP-1 was expressed in 61 cases of primary NPC (40.4%), 38.6% of recurrent NPC, and 45% of metastatic NPC, respectively. Wild type p53 protein was expressed in most NPC specimens, the results were 86.1% in primary, 84.1% in recurrent, and 95% in metastatic NPC. The bcl-2 protein was consistently expressed in NPC, 90% in primary, 86.7% in recurrent, and 100% in metastatic NPC. The bax was highly expressed in NPC, 94.7% in primary, 100% in recurrent, and 91% in metastatic NPC. The bcl-X protein was 91% in primary, 100% in recurrent, and 100% in metastatic NPC. About expression of drug resistance gene in NPC, low expression of MDR-1 was found in NPC (13.2% in primary, 25% in recurrent, and 0% in metastatic NPC) but highly expression of GST-π in NPC (57.6% in primary, 68.2% in recurrent, and 65% in metastatic NPC).

B. Detection of mRNA by Laser Capture Microdissection and Real-Time RT-PCR

Laser capture microdissection (LCM) method was used for dissecting tumor cells from frozen sections and paraffin sections of NPC biopsies for studying the expression of MDR-1,

MRP, GST-π, TS, p53, bcl-2, bax, bcl-$X/_L$, bcl-$X/_S$, the EBV proteins, such as LMP1, LMP1-TW, BHRF-1, Zebra, and EGFR, HER-2 and other HER family in tumor cells. In first year, these studies will be done in 5 each cases of primary, recurrent, and metastatic NPC. Unfortunately, these studies are still undergoing and no available data can be presented now. Immunohistochemical staining of these gene products on the tissue specimens and their correlation with the clinical response of the patients, will be performed.

Discussion

From the first year results of our project, we found, 40% of primary NPC tumor expressed EBV LMP-1, which is consistent with previous report. Although about 67% of primary NPC was reported to express LMP-1 in fresh tumor tissue by immunoprecipitation. While, the expression of LMP-1 in recurrent and metastatic NPC was not reported before. We found a little higher expression of LMP-1 in recurrent and metastatic NPC.

Apoptosis-related proteins including p53, bcl-2, bax, bcl-X are highly expressed in NPC tumor tissue. The expression of these proteins are independent and are not correlated each other.

MDR-1 is rarely expressed in primary NPC. The statistic analysis showed that the patients with MDR-1 expression had worse prognosis than those without MDR-1 expression. TS was expressed in 21.2% of primary, 30.8% of recurrent, and 35% of metastatic NPC.

In conclusion, in this work we tried to explore the correlation of the expression of apoptosis-related proteins, EBV LMP-1, drug resistance gene products, and EGFR, HER-2, and other HER family in tumor cells of NPC. So far, we had great achievement in the study. More advanced results in this study will be expected by continuing support for next two years from National Science Council (NSC). But, since only little budget for this project, the results will be limited.

參考文獻

1. Cheng AL, Su IJ, Chen YC, et al.: Characteristic clinicopathologic features of Epstein-Barr virus-associated peripheral T-cell lymphoma. Cancer 1993; 72: 909-16.
2. Cheng AL, Su IJ, Chen YC, et al.: Expression of p-glycoprotein and glutathione-s-transferase in recurrent lymphoma: The possible role of Epstein-Barr virus, immunophenotypes, and other predisposing factors. J Clin Oncol 1993; 11: 109-15.
3. Tsai ST, Jin YT, Su IJ: Expression of EBER1 in primary and metastatic nasopharyngeal carcinoma tissues using in situ hybridization. A correlation with WHO histologic subtypes. Cancer 1996; 77:231-6.
4. De-Vathaire F, Sancho-Carnier H, De-The H, et al.: Prognostic value of EBV markers in the clinical management of nasopharyngeal carcinoma (NPC): A multicenter follow-up study. Int J Cancer 1988; 42:176-81.
5. Yip TTC, Ngan RKC, Lau WH, et al.: A possible prognostic role of immunoglobulin-G antibody against recombinant Epstein-Barr virus BZLF-1 transactivator protein ZEBRA in patients with nasopharyngeal carcinoma. Cancer 1994; 74: 2414-24.
6. Dawson CW, Rickison AB, Young LS: Epstein-Barr virus latent protein inhibits human epithelial cell differentiation. Nature (London) 1990; 344:753-8.

7. Miller WE, Shelton Earp H, Raab-Traub N: The Epstein-Barr virus latent membrane protein induced expression of epidermal growth factor receptor. J Virol 1995; 69:4390-98.
8. Paine E, Scheiman RI, Baldwin AS Jr, Raab-Traub N: Expression of LMP1 in epithelial cells leads to the activation of a select subset of NF-kB/Rel family protein. J Virol 1995; 69:4572-6.
9. Kieff E: Epstein-Barr virus—increasing evidence of a link to carcinoma. (Editorial) New Engl J Med 1995; 333:724-6.
10. Mosialos G, Birkenbach M, Yalamanchili R, et al.: The Epstein-Barr virus transforming protein LMP1 engages signaling proteins for tumor necrosis factor family. Cell 1995; 80:389-99.
11. Beg AA, Baltimore D: An essential role for NF-κB in preventing TNF-α-induced cell death. Science 1996; 274:782-4.
12. van Antwerp AAJ, Martin SJ, Kafri T, et al.: Suppression of TNF-α-induced apoptosis by NF-κB. Science 1996; 274:787-9.
13. Wang CY, Mayo MW, Baldwin AS Jr.: TNF-and cancer therapy-induced apoptosis: Potentiation by inhibition of NF-κB. Science 1996; 274:784-7.
14. Hu LF, Chen F, Zhen QF, et al.: Differences in the growth pattern and clinical course of EBV-LMP1 expressions and non-expressing nasopharyngeal carcinomas. Eur J Cancer 1995; 31A: 658-60.
15. Beck WT, Dalton WS: Mechanism of drug resistance. In Cancer; principles and practice of oncology. edited by Devita Jr.VT, Hellman S, Rosenberg SA. 5th ed.: pp 418-512, Lippincott-Raven Publishers. 1997.
16. Dive C, Hickman JA: Drug-target interactions: only the first step in the commitment to a programmed cell death? Br J Cancer 1991; 64:192-6.
17. Schinkel AH, Borst P: Multidrug resistance mediated by p-glycoprotein. Semin Cancer Biol 1991; 2:213-26.
18. Dietel M: What's new in cytostatic drug resistance and pathology. Pathol Res Pract 1991; 187:892-905.
19. Chan HSL, Haddad G, Thorner PS, et al.: P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. N Engl J Med 1991; 325:1608-14.
20. Balddini N, Scotlandi K, Barbanit-Brodano G, et al.; Expression of p-glycoprotein in high-grade osteosarcoma in relation to clinical outcome. N Engl J Med 1999; 333:1380-5.
21. O'Brien ML, Tew KD: Glutathione and related enzymes in multidrug resistance. Eur J Cancer 1996; 2A(6): 967-78.
22. Nishio K, Nakamura T, Koh Y, et al.: Drug resistance in lung cancer. Cur Opinion Oncol 1999; 11:109-15.
23. Johnston PG, Fisher ER, Rochette HE, et al.: The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. J Clin Oncol 1994; 12:2640-7.
24. Johnston PG, Lenz HJ, Leichman CG, et al.: Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. Cancer Res 1995; 55:1407-12.
25. Yeh KH, Shun CT, Chen CL, et al.: High expression of thymidylate synthase is associated

with drug resistance of gastric carcinoma to high dose 5-fluorouracil-based systemic chemotherapy. Cancer 1998; 82:1626-31.
26. Eastman A: Activation of programmed cell death by anticancer agents: cisplatin as a model system. Cancer Cells 1990; 2:275-80.
27. Hickman JA: Apoptosis and chemotherapy resistance. Eur J Cancer 1996; 32A:921-6.
28. Fisher DE: Apoptosis in cancer therapy: Crossing the threshold. Cell 1994; 78:539-42.
29. Levine AJ, Momand J, Finlay CA: The p53 tumor suppressor gene. Nature 1991; 351:453-456.
30. Lowe SW: Cancer therapy and p53. Curr Opin Oncol 1995; 7:547-553.
31. Lowe SW, Bodis S, McClatchey A, et al.: P53 status and the efficacy of cancer therapy in vivo. Science 1994; 266:807-810.
32. Asa T, Borresen AL, Geisler S, et al.: Specific p53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. Nature Med 1996; 2:811-814.
33. Wattel E, Preudhomme C, Hecquat B, et al.: P53 mutations are associated with resistance to chemotherapy and short survival in hematological malignancies. Blood 1994; 84:3148-3157.
34. Benhatter J, Cerottini JP, Saraga E, et al.: P53 mutations as a possible predictor of response to chemotherapy in metastatic colorectal carcinomas. Int J Cancer 1996; 69:190-192.
35. Bardeesy N, Beckwith JB, Pelletier J: Clonal expansion and attenuated apoptosis in Wilms' tumors are associated wit p53 gene mutations. Cancer Res 1995; 55:215-9.
36. Reed JC: Double identity for proteins of the Bcl-2 family. Nature 1997; 387:773-776.
37. Brown R: The bcl-2 family of proteins. Bri Med Bulletin 1996; 53:466-77.
38. Sheu LF, Chen A, Meng CL, et al.: Analysis of bcl-2 expression in normal, inflamed, dysplastic nasopharyngeal carcinoma: association with p53 expression. Human Pathol 1997; 28:556-62.
39. Niedobitek G, Agathanggelou A, Barber P, et al.: P53 overexpression and Epstein-Barr virus infection in undifferentiated and squamous cell nasopharyngeal carcinomas. J Pathol 1993; 170:457-61.
40. Masuda M, Shinokuma A, Hirakawa N, et al.: Expression of bcl-2, p53, and ki-67 and outcome of patients with primary nasopharyngeal carcinomas following DNA-damaging treatment. Head & Neck 1998; 20:640-4.
41. Hackel PO, Zwick E, Prenzel N, Ullrich A: Epidermal growth factor receptors: critical mediators of multiple receptor pathways. Current Opinion Cell Biol 1999; 11:184-9.
42. Wells A: EGF receptor. Int J Biochem Cell Biol 1999; 31:637-43.
43. Alaouli MA, Paterson J, Al Moustafa AE, Yen L: The role of ErbB-2 tyrosine kinase receptor in cellular intrinsic chemoresistance: mechanisms and implications. Biochem Cell Biol 1997; 75:315-25.
44. Miller WE, Earp HS, Raab-Traub N: The Epstein-Barr virus latent membrane protein 1 induces expression of the epidermal growth factor receptor. J Virol 1995; 69:4390-98.
45. Miller WE, Cheshrine JL, Baldwin AS Jr, Raab-Traub N: The NPC derived C15 LMP1 protein confers enhanced activation of NF-kappa B and induction of the EGFR in epithelial cells. Oncogene 1998; 16:1869-77.
46. Zheng X, Hu L, Chen F, Christensson B: Expression of Ki67 antigen, epidermal growth factor receptor and Epstein-Barr virus-encoded latent membrane protein (LMP1) in nasopharyngeal

carcinoma. Eur J Cancer. Part B, Oral Oncology 1994; 30B:290-5.

47. Jin O, Chen S, Li G, Yao K: Expression of CerbB-2 and EGFR mRNA in human nasopharyngeal carcinomas and pericarcinomatous tissues. [Chinese] Bulletin of Hunan Med Univer 1997; 22:487-90.
48. Duan C, Chen Z, Zhao M: Effect of EBV-LMP and EGF autocrine on proliferation of nasopharyngeal carcinoma cell line CNE and their relationship in action. [Chinese] Bulletin of Hunan Med Univer 1997; 22:483-6.
49. Chao HH, Yang VC, Chen JK: Acidic FGF and EGF are involved in the autocrine growth stimulation of a human nasopharyngeal carcinoma cell line and sub-line cells. Int J Cancer 1993; 54:807-12.
50. Guan E, Zhou TC, Wang JH, et al.: Growth inhibition of human nasopharyngeal carcinoma in athymic mice by anti-epidermal growth factor receptor monoclonal antibodies. Int J Cell Cloning 1989; 7:242-56.
51. Sun Y, Fry DW, Vincent P, et al.: Growth inhibition of nasopharyngeal carcinoma cells by EGF receptor tyrosine kinase inhibitors. Anticancer Res 1999; 19:919-24.
52. Roychowdhury DF, Tseng A Jr., Fu KK, Weinburg V, Weidner N: New prognostic factors in nasopharyngeal carcinoma. Tumor angiogenesis and C-erbB2 expression. Cancer 1996; 77:1419-26.
53. Yu D, Liu B, Tan M, et al.: Overexpression of c-erbB-2/neu in breast cancer cells confers increased resistance to taxol via mdr-1-independent mechanisms. Oncogene 1996; 13:1359-65.
54. Tsai CM, Yu D, Chang KT, et al.; Enhanced chemoresistance by elevation of p185neu levels in HER-2/neu-transfected human lung cancer cells. J Natl Cancer Inst 1995; 87:682-4.
55. Pegram MD, Finn RS, Arzoo K, et al.: The effect of HER-2/neu overexpression on chemotherapeutic drug sensitivity in human breast and ovarian cancer cells. Oncogene 1997; 15:537-47.
56. Paik SM, Chung HC, Yang WI, et al.; Overexpression of erbB-2 in gastric cancer. Proc Am Assoc Cancer Res 1991; 32:291a.
57. Hancock MC, Langton BC, Chan T, et al.: A monoclonal antibody against the c-erbB-2.protein enhances the cytotoxicity of cis-diamminedichloroplatinum against human breast and ovarian tumor cell line. Cancer Res 1991; 51:4575-80.
58. Baselga J, Norton L, Albanell J, et al.: Recombinant humanized anti-HER2 antibody (HerceptinTM) enhances the anti-tumor activity of paclitaxel and doxorubicin against HER2/neu overexpression human breast cancer xenografts. Cancer Res 1998; 58:2825-31.
59. Bridges AJ: The rationale and strategy used to develop a series of highly potent, irreversible inhibitors of the epidermal growth factor receptor family of tyrosine kinases. Current Medicinal Chemistry 1999; 6:825-43.
60. Naselga J, Tripathy D, Mendelsohn J, et al.: Phase II study of weekly intravenous recombinant humanized anti-$p185^{HER2}$ monoclonal antibody in patients with NER-2/neu-overexpressing metastatic breast cancer. J Clin Oncol 1996; 14:737-44.
61. Cobleigh MA, Vogel CL, Tripathy D, et al.: Efficacy and safety of Herceptin^{TM} as a single agent in 222 women with HER2 overexpression who relapsed following chemotherapy for metastatic breast cancer [Abstract]. Proc Am Soc Clin Oncol 1998; 17:97a.

62. Vogel CL, Cobleigh MA, Tripathy D, et al.; Efficacy and safety of Herceptin^{TM} as a single agent in the first-line treatment HER2 overexpressing metastatic breast cancer (HER+/MBC) [Abstract]. Breast Cancer Res Treat 1998; 50:232.
63. Pegram MD, Lipton A, Hayes DF, et al.: Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185$^{HER2/neu}$ monoclonal antibody plus cisplatin in patients with HER2.neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J Clin Oncol 1998; 16:2659-71.
64. Slamon D, Leyland-Jones B, Shak S, et al.: Addition of Herceptin^{TM} (humanized anti-HER2 antibody) to first line chemotherapy for HER2 overexpressing metastatic breast cancer (HER+MBC) markedly increases anticancer activity: a randomized, multinational controlled phase II trial [Abstract]. Proc Am Soc Clin Oncol 1998; 17:98a.
65. Mendelsohn J: Epidermal growth factor receptor inhibition by a monoclonal antibody as anticancer therapy. Clin Cancer Res 1997; 3(12 Pt 2): 2703-7.

計劃成果自評

In first year of this 3-year study, we have made some progress on the planned 3 angles of drug resistance mechanism research of NPC tumor tissues. We plan to present part of our results to Annual Meeting of AACR next year.

Regarding the part of EBV and drug resistance of NPC, we expected that correlated the results of immunohistochemical study of EBV LMP-1 and drug resistance genes (MDR-1, GST-π, and TS) in primary, recurrent, and metastatic NPC will help us to find out the role of LMP-1 expression in mechanism of drug resistance of NPC. Also, we can correlate the results of apoptosis-related proteins, drug resistance genes, and LMP-1 and analyze statistically.

The modulation of HER1 by either monoclonal antibodies or specific HER1-tyrosine kinase inhibitor indeed raised the possibility of using this approach in treating NPC patients. We will further explore the effect of combining anticancer drugs and anti-HER1. The result of immunohistochemical study for expression of EGFR in NPC may provide possible therapeutic modality for recurrent and/or metastatic NPC in the near future.

Finally, further clinicopathologic correlation studies will continue to explore the possible roles of EBV-viral proteins, other drug resistance markers, and HER family members in terms of their significance of predicting the outcome of NPC patients receiving chemotherapy.