行政院國家科學委員會補助專題研究計畫成果報告

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※　　利用 UCN-01 來增進 E2F-1 蛋白分解及抗代謝※
※　　抗癌藥物所引起的細胞凋亡　　　　　　　　※
※　　(Enhancement of E2F-1 proteolysis and　　※
※　　antimetabolite-induced apoptosis by UCN-01)　※
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計畫類別：■個別型計畫□整合型計畫

計畫編號：NSC90-2314-B-039-007

執行期間：90 年 8 月 1 日至 91 年 7 月 31 日

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中華民國 91 年 12 月 26 日

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一、中文摘要

Thymidylate synthase (TS)及 dihydrofolate reductase (DHFR)分別是抗代謝抗癌藥物 5-fluorouracil (FU)及 methotrexate (MTX)的主要標記酵素。E2F-1 是在細胞生長週期 G1/S 轉換過程中很重要的一個基因轉錄因子，並可調控 TS 及 DHFR 的基因表達。我們發現在人類大腸癌細胞 HCT-116，UCN-01(一種抑制蛋白催化酵素丙型及細胞生長週期的臨床測試藥物)減少 TS、DHFR 及 E2F-1 的基因表達。UCN-01 可顯著地促進 FU及 MTX 所造成細胞凋亡的效果。另外在人類頭頸部癌細胞 KB 培養於含生理濃度的葉酸培養基中，每三至四週以高濃度及低濃度的 MTX 治療 24 小時，其最主要的抗藥機轉為增加 DHFR 基因表達及減少 MTX 的多重麩氨酸鹽作用(polyglutamation) 。UCN-01 可抑制 DHFR 蛋白表達於 KB 細胞，並可用來減弱癌細胞對 MTX 的抗藥性。

關鍵詞：抗癌藥物、細胞凋亡、細胞生長週期、抗藥性

Abstract

Thymidylate synthase (TS) and dihydrofolate reductase (DHFR) are the target enzymes for antimetabolites, 5-fluorouracil (FU) and methotrexate (MTX), respectively. Overexpression of either TS or DHFR contributes to poorer clinical outcome and drug resistance, and E2F-1 can upregulate both TS and DHFR expression through transcriptional activation. We have found in human colon cancer cell lines HCT-116, UCN-01, a protein kinase C/cyclin-dependent kinase inhibitor, can suppress TS, DHFR and E2F-1 gene expression. Additionally, UCN-01 can enhance FU and MTX-induced apoptosis in HCT-116. Human nasopharyngeal epidermoid carcinoma (KB) cells, adapted to long-term growth in folate-deficient medium containing physiological concentration of folate, 2 nM leucovorin, were exposed for 24 hr to either 3 or 30 ìM MTX for 6 cycles. After 6 cycles of MTX treatment, MTX polyglutamation was substantially decreased in KB cells exposed to 3 ìM MTX but to a much lesser extent in 30 μM MTX exposure group. There was an approximate 2.7-fold increase in the DHFR mRNA levels in KB cells exposed to 3 μM MTX. UCN-01 can suppress DHFR protein expression in KB cells and can enhance MTX-induced apoptosis. UCN-01 may be used to overcome drug resistance against MTX.

Keywords: chemotherapy, apoptosis, cell cycle, drug resistance

二、緣由與目的

TS and DHFR are target enzymes for antimetabolites, FU and MTX, respectively. Both FU and MTX have gained widespread clinical uses as important components of chemotherapeutic regimen of various

malignancies including gastric, colon and head and neck cancers.

The induction of apoptosis (programmed cell death) by FU and MTX is mainly dependent on the duration of inhibition of the target enzymes. Both TS and DHFR gene expression increases substantially during G1/S phase boundary of cell cycle. The transcription factor E2F-1 has been implicated in cell cycle control by regulating transcription of several genes required for DNA synthesis including DHFR and TS. The loss of functional pRb can give rise to increased free E2F-1 levels, subsequently increased levels of TS and DHFR and resistance to antimetabolites.

Most of the cytotoxic anticancer drugs in current use have been shown to induce apoptosis in susceptible cells. However, in clinical practice, the majority of solid tumors remain resistant to most chemotherapeutic agents. It is becoming increasingly clear that the most important determinant of tumor resistance may be a generalized resistance to induction of apoptosis. By identifying the anti-apoptotic/apoptotic signals and perturbing the signal transduction for anti-apoptosis, it is possible to overcome this form of drug resistance.

三、結果與討論

We have previously shown that UCN-01, a protein kinase C and cyclin-dependent kinase inhibitor, can suppress TS expression in human gastric cancer cells SK-GT5 by increasing ubiquitinization of E2F-1. In current study, we have found that in human colon cancer cell line, HCT-116, UCN-01 can suppress E2F-1, TS and DHFR expression by Western blotting. By using quantitative fluorescent microscopy and terminal deoxynucleotidyl transferase assays, we have found that UCN-01 can enhance FU and MTX-induced apoptosis in HCT-116 cells to approximate 50% from less than 5% when using FU or MTX alone. This confirms the data from SK-GT5, and can be used to design the combination clinical trial in gastrointestinal malignancy.

Then we examined the resistance mechanism against MTX in human KB cells, a head and neck cancer cell line, adapted to growth in physiological concentration of folate. We used 24-h exposure of high- and low-dose (30 and 3 μM MTX) every 3 to 4 weeks for 6 cycles. After 6 cycles of MTX treatment, MTX polyglutamation was substantially decreased in KB cells exposed to 3 ìM MTX but to a much lesser extent in 30 μM MTX exposure group. There was an approximate 2.7-fold increase in the DHFR mRNA levels in KB cells exposed to 3 μM MTX. UCN-01 can suppress DHFR protein expression in KB cells, and when used in combination with MTX can cause more than 60% apoptosis compared to less than 5% by either drug. UCN-01 can be used to overcome drug resistance against MTX and enhance MTX-induced apoptosis.

四、計畫成果自評

We have accomplished most of our aims. Though some of the results need more investigative works, we are expecting at least 1 SCI-grade publications from this project. Further study is needed to characterize the regulatory role of UCN-01 on E2F-1,

and their inter-relationship.

五、參考文獻

Horikoshi, T., Danenberg, K. D., Stadlbauer, T. H., Volkenandt, M., Shea, L. C., Aigner, K., Gustavsson, B., Leichman, L., Frosing, R., Ray, M., Gibson, N. W., Spears, C. P., and Danenberg, P. V. Quantitation of thymidylate synthase, dihydrofolate reductase, and DT-diaphorase gene expression in human tumors using the polymerase chain reaction. Cancer Res., *52:* 108-116, 1992.

Goker, E., Waltham, M., Kheradpour, A., Trippett, T., Mazumdar, M., Elisseyeff, Y., Schnieders, B., Steinherz, P., Tan, C., Berman, E., et al. Amplification of the dihydrofolate reductase gene is a mechanism of acquired resistance to methotrexate in patients with acute lymphoblastic leukemia and is correlated with p53 gene mutations. Blood, *86:* 677-684, 1995.

Matherly, L. H., Taub, J. W., Ravindranath, Y., Proefke, S. A., Wong, S. C., Gimotty, P., Buck, S., Wright, J. E., and Rosowsky, A. Elevated dihydrofolate reductase and impaired methotrexate transport as elements in methotrexate resistance in childhood acute lymphoblastic leukemia. Blood, *85:* 500-509, 1995.

Hsueh, C.-T.; Wu, Y.-C.; Schwartz, G.K. UCN-01 suppresses E2F-1 mediated by ubiquitin-proteasome-dependent degradation. Clin. Cancer Res. 7:669-674, 2001.

Pizzorno, G.; Mini, E.; Coronnello, M.; McGuire, J.J.; Moroson, B.A.; Cashmore, A.R.; Dreyer, R.N.; Lin, J.T.; Mazzei, T.; Periti, P. and Bertino, J.R. Impaired polyglutamylation of methotrexate as a cause of resistance in CCRF-CEM cells after short-term, high-dose treatment with this drug. Cancer Res., *48*: 2149-2155, 1988.

van der Laan, B F.; Jansen, G; Kathmann, I.; Schornagel, J.H. and Hordijk, J. Mechanisms of acquired resistance to methotrexate in a human squamous carcinoma cell line of the head and neck, exposed to different treatment schedules. Eur. J. Cancer, *27*: 1274-1278, 1991.