行政院國家科學委員會專題研究計畫　期中進度報告

皮質類固醇影響上皮細胞癌生長及化學藥物敏感性機轉之研究並探討與癌細胞反應模式相關之分子分類(2/3)

計畫類別：個別型計畫
計畫編號：NSC92-2314-B-002-105-
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內科

計畫主持人：鄭安理

計畫參與人員：盧彥伸

報告類型：精簡報告
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 5 月 31 日

行政院國家科學委員會專題研究計畫期中進度報告

皮質類固醇影響上皮細胞癌生長及化學藥物敏感性機轉之研究並探討與癌細胞反應模式相關之分子分類

Studies on the mechanisms of glucocorticoids on the growth and drug sensitivity of carcinomas, and exploring relevant molecular classification

一、中文摘要

皮質類固醇除本身對於某些血液腫瘤具細胞毒性之外，也常與抗癌化學藥物併用以治療因化學藥物引起之噁心、嘔吐及過敏反應等副作用。雖然類固醇已被証實可以影響多種細胞之重要訊息傳遞徑路，其中有些與癌細胞抗藥性有關。然而我們對類固醇類藥物對於與一般癌細胞生長以及化學藥物感受性可能產生之影響仍所知極少。釐清這個問題對臨床腫瘤治療將會有重要影響。

在我們先前的研究中，我們隨機選擇了十四株癌細胞株有系統地進行研究以解答這個問題。Dexamethasone（DEX）被選為皮質類固醇代表藥物。我們已發現：DEX 確實對癌細胞株（十四株之中的七株）的生長以及化學藥物感受性有影響。DEX 對癌細胞的影響呈現異質性而且似乎是彼此互斥的。且此影響是皮質類固醇受體—依賴性的。在我們今年的進一步研究中我們發現

1. 為了尋找可能造成 DEX 對癌細胞株的生長以及化學藥物感受性異質性影響的機轉或決定性因子(determinant factor)，我們研究皮質類固醇受體共同協調因子(steroid receptor co-regulator)在這些細胞的表現。我們發現這些對 DEX 有著不同反應的癌細胞中皮質類固醇受體共同協調因子(steroid receptor co-regulator)的表現各有不同。而且其中有許多共同協調因子之表現量本身亦受到皮質類固醇受體所影響。
2. 利用即時定量 RT-PCR 測定，以及利用免疫組織化學染色檢驗一百多位病患的(肺癌、乳癌、子宮頸癌)癌細胞檢體我們發現在不同病人的癌細胞中的皮質類固醇受體含量有高有低。我們推測臨床上有一定比例之癌細胞含有高濃度皮質類固醇受體，並可能對 DEX 有感受性。
3. 在我們先前的研究發現 DEX 在 SiHa 細胞所造成的化學藥物致敏感效應與其對 NF-kB 的調控有著高度相關。透過轉殖含有 dominant negative IkB 的 plasmid 進入 SIHa cells 中以抑制 NFkB 活性，我們發現原先 DEX 提高 SiHa 細胞對 cisplatin 的化學藥物感受性的現象消失。而在我們進一步的研究發現在 SiHa 細胞中皮質類固醇受體確實會與 NFkB 有直接作用(dirrect protein-protein interaction)。
4. 我們另外發現 DEX 會直接作用於 MCF7 細胞的 p21 promoter 上造成 p21 的向上調控，表現量增加進而造成細胞週期 G1 arrest, 抑制癌細胞的生長。

關鍵詞：皮質類固醇、癌細胞、化學藥物感受性

Abstract

Objectives: Glucocorticoids (GCs) are commonly co-administered with anti-cancer drugs such as cisplatin to prevent drug-induced allergic reaction, nausea, and vomiting. But little is known regarding the effects of GCs on the growth and chemosensitivity of common carcinomas cells. In our previous study, we have demonstrated that DEX had mutually

exclusive effects on either growth or cisplatin sensitivity in 7 of the 14 cell lines. DEX inhibited cell growth of 4 (MCF-7, MCF-7/MXR1, MCF-7/TPT300, and HeLa), increased cisplatin cytotoxicity of one (SiHa), and decreased cisplatin cytotoxicity of 2 (H460 and Hep3B) cells lines. Although the effect of DEX on these carcinoma cells was unexpectedly diverse, it remained GC receptor (GCR) dependent. In this study, we further explore the mechanism and the determinant factor on the diverse effect of DEX effect on the cancer cells. ***Methods:*** Twenty one steroid receptor co-regulators were examined by RT-PCR in these 14 cancer cell lines. Chromosome immuno-precipitate with anti-GCR antibody and then PCR for the promoter area of these co-regulators was performed. Quantitative RT-PCR for GCR and immunohistochemico stain for GCR was performed in breast, lung and cervical cancer patient's tumor specimens. ***Results:*** The expressions of the steroid receptor co-regulators of these cells are examined. Correlation with the difference between expression of the coregulators and DEX responsiveness were also examined. Furthermore, the expressions of some of the co-regulators were influenced by the GCR. In human cancer samples, we demonstrated that some of the breast, lung and uterine cervical cancer do express high level of GCR. In MCF7 cells, we found DEX induced p21 up-regulation and caused G1 phase arrest of MCF-7. Addition of excess amounts of a structure homologue of DEX, RU486, completely abolished the growth suppression effect of DEX, suggesting that DEX act via GCR-related signal transduction pathways. Furthers, DEX has no effect on the growth of MCF-7/GCR(-), an MCF-7 subclone contains vary low levels of GCR ($<1x10^3$/cell). Compared with MCF-7, MCF-7/GCR(-) contains no detectable level of CBP300, HDAC1, and significantly lower levels of NCOR1, TIF2, GCN5L2, and ARA70. Transfection of GCR RNAi to MCF-7 cells also resulted in no detectable level of CBP300, HDAC1, and significantly lowers levels of NCOR1, TIF2, GCN5L2, and ARA70. Transfection of human GCR to MCF-7/GCR(-) restored the expression of GCR and all these co-regulators and sensitivity to DEX in MCF-7/GCR(-) cells. Chromosome IP with anti-GCR antibody and PCR study showed positive result with TIF-2, imply the possibility of direct regulation of TIF-2 expression by GCR. In SiHa cells, we demonstrated that the The cytotoxicity-enhancing effect of GC in SiHa cells correlated well with its effect on abrogating the cisplatin-induced activation of NF-κB. Expression of a dominant-negative truncated IκBα gene in SiHa cells completely abolished the cytotoxicity-enhancing effect of DEX. **Conclusions:** GCs may affect growth or chemosensitivity of carcinoma cells containing high concentration of functional GCR. Although the effects are heterogeneous and currently unpredictable, our data underscore the importance of clarifying the impact on tumor control by the co-administed GCs to carcinoma patients receiving chemotherapy. It is mandatory to identify the molecular and cellular markers that help predict the diverse effect of GCs on carcinoma cells.

Keywords: Glucocorticoids, Glucocorticoid receptor, Carcinoma. Cell growth, Chemosensitivity, Drug resistance.

二、緣由與目的

Although GCs are effective in inducing apoptosis via yet uncharacterized pathways in many hematological malignancies [1, 2, 3], they are generally not effective in the treatment of non-hematological solid tumors. However, in such tumors, co-administration of GC with anti-cancer drugs is a common clinical practice to prevent drug-induced allergic reaction or nausea/vomiting. Although GCR is ubiquitous in cancer cells and GCR has been linked to signal transduction pathways pertinent to their growth, defense, and apoptosis [4, 5, 6], little is known regarding the effects of GC on the growth and chemosensitivity of common human carcinomas. Several studies have shown diverse effects of GC on chemosensitivity in non-hematological neoplastic cells. Wolff et al. reported that DEX induced drug resistance toward

cisplatin in C6 glioma cells [7]. Weller et al. also reported a DEX-mediated cytoprotection in glioma cell lines [8, 9]. However, Benckhuijsen et al. reported an enhancement of melphalan cytotoxicity by DEX in melanoma cells [10]. A more comprehensive study is needed to clarify the role of GC on chemosensitivity of non-hematological neoplastic cells.

In our previous study, we have demonstrated that DEX had mutually exclusive effects on either growth or cisplatin sensitivity in 7 of the 14 cancer cell lines. DEX inhibited cell growth of 4 (MCF-7, MCF-7/MXR1, MCF-7/TPT300, and HeLa), increased cisplatin cytotoxicity of one (SiHa), and decreased cisplatin cytotoxicity of 2 (H460 and Hep3B) cells lines. Although the effect of DEX on these carcinoma cells was unexpectedly diverse, it remained GC receptor (GCR) dependent. The GCR contents of the 7 cell lines affected by DEX were significantly higher than those of the other 7 cell lines unaffected by DEX ($5.2\pm2.5 \times 10^4$ vs $1.3\pm1.4\times10^4$, P=0.005).Only two DEX-unresponsive cell lines (NPC-TW01 and NPC-TW04) had GCR contents at the high range as those of the 7 DEX-responsive cell lines. On further examination, the function of the endogenous GCR of these two cell lines was found to be impaired. Further, transfection and expression of a vector encoding GCR to AGS, a GCR low-expressing and GC non-responsive cell line, increased its susceptibility to DEX manifested as an increased resistance toward cisplatin.

In this study, we further explore the mechanism and the determinant factor on the diverse effect of DEX effect on the cancer cells.

三、方法

1. Fourteen cancer cell lines were tested, including: SiHa cells (human cervical carcinoma), HeLa, Caski cells (human cervical carcinoma), H460 cells (human lung carcinoma), Hep3B, Hut 7 cells (human hepatocellular carcinoma), and MCF-7 cells (human breast cancer) MCF-7/MXR1 and MCF-7/TPT300 cells, NPC-TW01 and NPC-TW04 cells (nasopharyngeal cancer) AGS, N87, and SNU1 cells (human gastric cancer).
2. Growth inhibition was estimated by MTT assay
3. The expression of 27 coregulators were examined by RTPCR. Immunohistochemical stain for GCR and
4. The effects of DEX on cell cycle phases and cell cycle regulators were examined by Western blot and flow cytometry study.
5. Transfection of GCR RNAi expression vector into MCF-7 and transfection of GCR-Expressing Vector into MCF-7/GCR(-) by Lipofectamine 2000
6. Chromatin immuno-precipitate with anti-GCR antibody and then PCR for CBP300, HDAC1, NCOR1, TIF2, GCN5L2, and ARA70.
7. The physical interaction between GCR and NFkB were examined by immunoprecipitate assay.

四、結果

Expression Profiles of the Co-regulators the 14 Carcinoma Cell Lines

In order to explore the mechanism and the determinant factor on the diverse effect of DEX effect on the cancer cells, we examined the expression of 21 steroid receptor co-regulators in these 14 carcinoma cell lines. The 14 carcinoma cancer cells represented 4 groups of different DEX responses; including growth inhibitory, increase chemsensitivity, decrease chemosensitivity, and DEX non-responsive. The co-regulators including: coactivators: SRC1, (NCOA1), TIF2a (NCOA2), AIB1 (NCOA3), ARA70 (NCOA4), ARA54 (RNF14), TIF1, CARM1, SRCAP P300 (EP300), CBP (CREBBP), GCN5L2, PCAF, BRG1(SMARCA4), hBRM (SMARCA2), SNF5 (SMARCB1),

BAF60b (SMARCD2), Corepressors: NCOR1, SMRT (NCOR2), REA, HDAC1, HDAC2, HDAC3, SIN3B (KIAA0700), SAP18, SAP30, MTA1, MTA1L1. We found that the expression levels of these co-regulators are different in different groups of cancer cells. The correlation of these different expressions in co-regulator there the diverse DEX responsiveness are under further investigation. We also found that the expression of some of the co-regulator is regulated by the GCR. The detail pathway of the regulation is under investigation.

Expression of GCR in Carcinoma Cell Lines and Human Cancer Tumor Samples

We examine the expression of GCR in human carcinoma samples. Immunohistochemical staining for GCR were performed in over 130 tissue samples from human breast, lung and uterine cervical cancer tissues. And the real-time RT PCR was performed in 20 of these samples to validate the quantitation of GCR expression, especially in comparison with the carcinoma cell lines that have been tested in our previous studies. We found that the 5 of the 42 breast cancer tissue sample and 30 of the 74 non-small cell lung cancer and 14 of 20 cervical cancer tissue samples express very high level of GCR. In non-small cell lung cancer, the expression level of GCR has no correlation with the age, gender, cell type(adenocarcinoma or squamous cell carcinoma). Real-time RT-PCR of TBP mRNA showed the integrity of the tumoral RNA to be good. GCR mRNA expression in breast cancer tissue (standardized to TBP mRNA) was expressed as -[DELTA] C_T = -($C_{T\ GCR}$ –$C_{T\ TBP}$). The level of GCR mRNA expression correlate well with the GCR number measured by [^{3}H] DEX ligand biding assay.

Glucocorticoids (GC) enhance p21 expression and induce G1 phase cell cycle arrest in MCF-7 cells. The possible role of GC receptors and their co-regulator

We found that dexamethasone (DEX), at clinically achievable concentration (0.01-1.0μM) inhibits cell growth of MCF-7 cells while induces no cellular apoptosis.

DEX induced p21 up-regulation and caused G1 phase arrest of MCF-7. Addition of excess amounts of a structure homologue of DEX, RU486, completely abolished the growth suppression effect of DEX, suggesting that DEX act via GCR-related signal transduction pathways. Furthers, DEX has no effect on the growth of MCF-7/GCR(-), an MCF-7 subclone contains vary low levels of GCR (<1x10^3/cell). Compared with MCF-7, MCF-7/GCR(-) contains no detectable level of CBP300, HDAC1, and significantly lower levels of NCOR1, TIF2, GCN5L2, and ARA70. Transfection of GCR RNAi to MCF-7 cells also resulted in no detectable level of CBP300, HDAC1, and significantly lowers levels of NCOR1, TIF2, GCN5L2, and ARA70. Transfection of human GCR to MCF-7/GCR(-) restored the expression of GCR and all these co-regulators and sensitivity to DEX in MCF-7/GCR(-) cells. Chromosome IP with anti-GCR antibody and PCR study showed positive result with TIF-2, imply the possibility of direct regulation of TIF-2 expression by GCR.

Direct Physical Interaction of GCR and NF-kB may Inhibit NF-κB Activation and Blocks the Cytotoxicity-enhancing Effect of DEX in SiHa Cells

In our previous study, we have demonstrated that DEX Suppressed Cisplatin-induced NF-κB Activation in SiHa Cells. To further examine the role of NF-κB in the cytotoxicity-enhancing effect of DEX, we generated a re-combinant plasmid containing dominant negative IκBα (dnIκBα) gene. This dnIκBα protein does not contain the residues necessary for signal-induced phosphorylation and proteasome-mediated degradation of IκBα, thereby preventing dissociation and translocation of NF-κB to the nucleus. The expression of the dnIκBα in pooled stably transfected SiHa cells was verified by Western blot analysis. The

control-pRCMV-transfected SiHa cells contained only the endogenous wild-type IκBα protein, while the dnIκBα-pRCMV-transfected SiHa cells contained an additional band representing the truncated exogenous IκBα protein. Results of EMSA showed that NF-κB binding activity was markedly suppressed in the dnIκBα-pRCMV-transfected cells after either TNF-α or cisplatin treatment. In addition, the cytotoxicity-enhancing effect of DEX in dnIκBα-pRCMV-transfected SiHa cells was abolished. The dnIκBα-pRCMV-transfected SiHa cells had also become more sensitive to cisplatin as compared to the control-pRCMV-transfected SiHa cells. These data confirmed that NF-κB plays a central role in the chemosensitizing effect of DEX in SiHa cells. To test whether the inhibition of NFkB activity were through activation of IKB by the GCR or the direct physical protein-protein interaction, we examined the expression of the IKB in SiHa cell under different time points of DEX treatment. We also examined the protein-protein interaction by immunoprecipitation study. We found that the expression of IkB was not influenced by the treatment of DEX. However, the immunoprecipitate study did show that the GCR physically interact with the NFkB in SiHa cells. The histidine tag pull-down assay showed that there is interaction between the C-terminal of GCR and the NFkB.

四、討論

In the present study, we examined the mechanism DEX on the chemosensitivity of 14 carcinoma cell lines and steroid receptor co-regulator expression in these cell lines. We found that GC exerted a GCR-related differential effect on the growth or chemosensitivity of the majority of carcinoma cells. The co-regulator profiles were also explored. The results of this study indicate that, while GC may be co-administered with anti-cancer drugs for other reasons, the possible effect of GC on the chemosensitivity of some selected cancers may be clinically significant and requires further investigation.

This study has demonstrated that GC affects either growth or chemosensitivity in a substantial portion of carcinoma cells. Since GCs are commonly co-administered with anticancer drugs such as taxanes and platinums, how GC alter the effect of chemotherapy may have to be taken into consideration in clinical practice. As shown in this study, it may not be difficult to identify those carcinoma patients of whom tumor response is going to be affected by GC, since only cells with high GCR content are affected. However, how GC will actually affect the growth of tumors of these cancer patients remains uncertain since the effects of GC, as disclosed in this study, are extremely diverse and without useful cellular or molecular predictors.

A direct correlation between GCR content of the cells and the magnitude of physiologic response to GC has been reported in hematologic malignancies[20]. The sensitivity of many lymphoid cell lines to GC-induced growth arrest and apoptosis is directly proportional to intracellular receptor content [21-23]. Several studies also identified a correlation between reduced GCR expression and a poor treatment response as well as poor prognosis in patients with acute lymphocytic leukemia, suggesting that reduced GR expression could lead to clinical glucocorticoid resistance [19, 24-26]. Our study demonstrated that the susceptibility to the effect of DEX on cell growth or chemosensitivity in carcinoma cells is also correlate well with the level of GCR content. However, the GCR contents of the GC-responsive carcinoma cells are almost 10 times higher than that of lymphoid cells [24-26], suggesting that the cellular contexts or the signal transduction pathways for the interaction of GC and GCR are probably different between these two groups of cells.

The expressions of the GCR have not yet been examined in cancer tissues. In this study, we examined the expression of GCR by the immunohistochemical stain and quantitative RT-PCR in human cancer samples. The expression level between the result of immunohistochemical stain and quantitative RT-PCR were correlated well. We found that some of the human cancer tissues do express high level of GCR. Therefore, the clinical impact of DEX co-treatment with chemotherapy in these cancer patients should not be overlooked.

Activated GCR may activate or suppress gene expression through interaction with respective positive or negative *cis*-acting regulatory elements in the promoters regions [27, 28]. Activated GR can also regulate the expression of GC responsive genes indirectively through protein-protein interactions with other transcription factors such as NF-κB and AP-1 [29–30]. Inactivation of NF-κB or AP-1 has been shown to alter the vulnerability of cancer cells to several cytotoxic agents [31, 32]. Activation of NF-κB has been implicated in mediating drug resistance of cancer cells. NF-κB could be activated by a variety of stresses, including oxidative stress and DNA damage (33-36). Activated NF-κB may prevent the triggering of apoptosis, and thus result in drug resistance against DNA-damaging agents (37-40). The molecular mechanism of NF-κB-mediated protection of cells remains unclear, but may involve the up-regulation of caspase inhibitors (41). In this study, we have provided evidence that NF-κB plays an important role in mediating the drug resistance of SiHa cells. Suppression of NF-κB activity by dnIκBα not only abolished the chemosensitizing effect of DEX, but also increased the chemosensitivity of SiHa cells to cisplatin. DEX had no effect on cells without discernable changes of NF-κB. Furthermore, we demonstrated that the GCR C-terminal is physically interacted with the NFkB. However, it remains to be clarified why certain carcinoma cell lines do not have an NF-κB response to cisplatin or have an NF-κB response which cannot be attenuated by DEX.

Our preliminary data have indicated that suppression of NF-κB is one of the major mechanism of interacting cisplatin sensitivity in SiHa cell. However, it remains difficult to explain the diverse effect of GC on GCR-rich carcinoma cells [42]. Although high-GCR content is necessary for a response of the cells to GC, the diversified and even contradictory effects of GC on these cells cannot be simply explained by the amount of GCR. Our findings suggest that an upstream switch point at the level of GC-GCR interaction may work to segregate the direction of downstream pathways. Recent studies on the action of androgen receptor (AR) may provide a possible example for the diverse effects of GC on carcinoma cells. Co-regulators of AR have played crucial roles in determining the ultimate activity of AR, and the presence or absence of certain co-regulators may even change the activity of anti-androgens to become androgens [43, 44]. Several novel co-regulators of GC have also been found to play important roles in the signaling pathway of the GCR [45]. The possibilities that the specific presence of certain co-regulators of GCR in different carcinoma cells may dictate the ultimate effect of GC need to be clarified. In this study, we have examined the expression of the steroid

After a single oral dose of 7.5 mg of DEX, the serum concentration of DEX was found to be around 0.12 μM lasting for 1~3 hours [46]. However, the serum concentration of DEX may reach 2 μM after a single intravenous infusion of 80~100 mg of DEX [47]. Since the administration of relatively high-dose DEX, at the range of 10-50 mg/day, or its equivalents, is widely used for the prevention of cisplatin-induced nausea/vomiting and taxanes-induced allergic reactions, the possible effect of GC on the chemosensitivity of some cancer patients needs to be seriously considered. In summary, the results of this study suggest that GC exerts a GCR-dependent effect on the growth

or chemosensitivity in a substantial portion of carcinoma cells. The clinical relevance and the cellular mechanisms that dictate the disparate effects of GC need to be further clarified.

五、計畫成果自評

In the present study, we examined the effects and mechanisms of DEX on the chemosensitivity of 14 carcinoma cell lines. We also explored the expression profiles of steroid co-regulators in these cells, and examined the expression of GCR in human cancer tissue samples. The results of this study indicate that, while GC may be co-administered with anti-cancer drugs for other reasons, the possible effect of GC on the chemosensitivity of some selected cancers may be clinically significant and requires further investigation.

The current results have beeen submitted for publication in the peer-reviewed journal.

六、參考文獻

1. Haskell, C. M. Antineoplastic agents. *In:* Haskell, C. M., Break, J. S.(eds.), Cancer Treatment, forth edition, pp. 105-106. Philadelphia: W. B. Saunders, 1995.
2. Wood, A. C., Waters, C. M., Garner, A., and Hickman, J. A. Changes in c-myc expression and the kinetics of dexamethasone-induced programmed cell death and apoptosis in human lymphoid leukemia cells. Br. J. Cancer, *69:* 663-669, 1994.
3. Schwartzman, R. A. and Cidlowski, J. A. Mechanism of tissue-specific induction of internucleosomal deoxyribonucleic acid cleavage activity and apoptosis by glucocorticoids. Endocrinology, *133:* 591-599, 1993.
4. Wright, A. P., Zilliacus, J., McEwan, I. J., Dahlman-Wright, K., Almlof, T., and Carlstedt-Duke, J. Structure and function of the glucocorticoid receptor. J. Steroid Biochem. Mol. Biol., *47:* 11-19, 1993.
5. Schwartzman, R. A. and Cidlowski, J. A. Glucocorticoid-induced apoptosis of lymphoid cells. Int. Arch. of Allergy Immunol., *105:* 347-354, 1994.
6. McEwan, I. J., Wright, A. P., and Gustafsson, J. A. Mechanism of gene expression by the glucocorticoid receptor: role of protein-protein interactions. Bioessays, *19:* 153-160, 1997.
7. Wolff, J. E., Denecke, J., and Jurgens, H. Dexamethasone induces partial resistance to cisplatinum in C6 glioma cells. AntiCancer Res., *16:* 805-809, 1996.
8. Weller, M., Schmidt, C., Roth, W., and Dichgans, J. Chemotherapy of human malignant glioma: prevention of efficacy by dexamethasone? Neurology, *48:* 1704-1709, 1997.
9. Naumann, U., Durka , S., and Weller , M. Dexamethasone-mediated protection from drug cytotoxicity: association with p21 WAF1/CIP1 protein accumulation? Oncogene, *17:* 1567-1575, 1998.
10. Benckhuijsen, C., Osman , A. M., Hillebrand, M. J., and Smets, L. A. "Glucocorticoid effect on melphalan cytotoxicity, cell-cycle position, cell size, and [3H]uridine incorporation in one of three human melanoma cell lines". Cancer Res., *47:* 4814-4820, 1987.
11. Yang, C. H., Schneider, E., Kuo, M. L., Volk, E. L., Rocch, E., and Chen, Y. C. BCRP/MXR/ABCP expression in topotecan-resistant human breast carcinoma cells. Biochem. Pharmacol., 60: 831-837, 2000.
12. Lin, C.T., Wong, C.I., Chan, W.Y., Tzung, K.W., Ho, J. K. C., Hsu, M.M. & Chuang, S.M. (1990). Establishment and characterization of two nasopharyngeal carcinoma cell lines. Lab. Investig. 62, 713-724.
13. Lin, C.-T., Chan, W.-Y., Chen, W., Huang, H.-M., Wu, H.-C., Hsu, M.-M., Chuang, S.-M. & Wang, C.-C.(1993). Characterization of seven newly established nasopharyngeal carcinoma cell lines. Lab. Investig. 68, 716-727.
14. Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., and Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res., *47:* 936-942, 1987.

15. Harmon, J. M. and Thompson, E. B. Isolation and characterization of dexamethasone-resistant mutants from human lymphoid cell line CEM-CT. Mol. Cell Biol., *1:* 512-521, 1981.16
16 Scheidereit, C., Geisse, S., Westphal, H. M., and Beato, M. The glucocorticoid receptor binds to defined nucleotide sequences near the promoter of mouse mammary tumour virus. Nature 304: 749-752, 1983.
17. Staal, F. J., Roederer, M., Herzenberg, L. A., and Herzenberg, L. A. Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA, *87:* 9943-9947, 1990.
18. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254, 1976.
19. Lippman, M. E., Yarbro, G. K., and Leventhal, B. G. Clinical implications of glucocorticoid receptors in human leukemia. Cancer Res., 38: 4251-4256, 1978.
20. Vanderbilt, J. N., Miesfeld, R., Maler, B. A., and Yamamoto, K. R. Intracellular receptor concentration limits glucocorticoid-dependent enhancer activity. Mol. Endocrinol., *1:* 68-74, 1987.
21. Gehring, U., Mugele, K., and Ulrich, J. Cellular receptor levels and glucocorticoid responsiveness of lymphoma cells. Mol. Cell Endocrinol., *36:* 107-113, 1984.
22. Bourgeois, S. and Newby, R. F. Diploid and haploid states of the glucocorticoid receptor gene of mouse lymphoid cell lines. Cell, *11:* 423-430, 1977.
23. Chapman, M. S., Askew, D. J., Kuscuoglu, U., and Miesfelt, R. L. Transcritional control of steroid-regulated apoptosis in murine thymoma cells. Mol. Endocrinol., *10:* 967-978, 1996.
24.Bloomfield, C. D., Smith, K. A., Peterson, B. A., and Munck, A. Glucocorticoid receptors in adult acute lymphoblastic leukemia. Cancer res., *41:* 4857-4860, 1981.
25. Pui, C. H., Dahl, G. V., Rivera, G., Murphy, S. B., and Costlow, M. E. The relationship of blast cell glucocorticoid receptor levels to response to single-agent steroid trial and remission response in children with acute lymphoblastic leukemia. Leuk. Res., *8:* 579-585, 1984.
26. Pui, C. H., and Costlow, M. E. Sequential studies of lymphoblast glucocorticoid receptor levels at diagnosis and relapse in childhood leukemia: an update. Leuk. Res. *10:* 227-229, 1986.
27. Wells, R.J., Mascaro, K., Young, P. C., and Cleary, R. E. Glucocorticoid receptors in the lymphoblasts of patients with glucocorticoid-resistant childhood acute lymphoblastic leukemia. Am. J. Pediatr. Hematol. Oncol., *3:* 259-264, 1981.
28. Powers, J. H., Hillmann, A. G., Tang, D. C., and Harmon, J. M. Cloning and expression of mutant glucocorticoid receptors from glucocorticoid-sensitive and glucocorticoid-resistant human leukemic cells. Cancer Res., 53: 4059–4065, 1993.
29. Reichardt, H. M., and Schutz, G. Glucocorticoid signalling–multiple variations of a common theme. Mol. Cell. Endocrinol., 146: 1–6, 1998.
30. McEwan, I. J., Wright, A. P. H., and Gustafsson, J-Å. Mechanism of gene expression by the glucocorticoid receptor: role of protein-protein interactions. Bioessays, 19: 153–160, 1997.
31. Dumont, A., Hehner, S. P., Schmitz, M. L., Gustafsson, J-Å., Liden, J., Okret, S., van der Saag, P. T., Wissink, S., van der Burg, B., Herrlich, P., Haegeman, G., De Bosscher, K., and Fiers, W. Cross-talk between steroids and NF-k: what language? Trends Biochem. Sci., 23: 233–235, 1998.
32. Gottlicher, M., Heck, S., and Herrlich, P. Transcriptional cross-talk, the second mode of steroid hormone receptor action. J. Mol. Med., 76: 480–489, 1998.
33. Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R., and Verma, I. M. Suppression of TNF-alpha-induced

apoptosis by NF-kappa B. Science, *274:* 787-789, 1996.
34. Sen, C. K. and Packer, L. (1996) Antioxidant and redox regulation of gene transcription. FASEB J. 10, 709-720.
35. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) DNA repair and mutagenesis. pp. 14-19. ASM Press: Washington D.C.
36. Bertoncini, C. R. and Meneghini, R. (1995) DNA strand breaks produced by oxidative stress in mammalian cells exhibit 3'-phosphoglycolate termini. Nucleic Acids Res. 23, 2995-3002.
37. Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R., and Verma, I. M. (1996) Suppression of TNF-alpha-induced apoptosis by NF-kappa B. Science 274, 787-789.
38. Beg, A. A. and Baltimore, D. (1996) An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. Science 274, 782-784.
39. Baringo M. (1996) A life-death balance within the cell. Science 274, 724.
40. Wang, C. Y., Mayo, M. W., and Baldwin, A. S. (1996) TNF-alpha and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. Science 274, 784-787.
41. Chu, Z. L., McKinsey, T. A., Liu, L., Gentry, J. J., Malim, M. H., and Ballard, D. W. (1997) Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-kappaB control. Proc. Natl. Acad. Sci. USA 94, 10057-10062.
42. Lu, Y. S., Yeh, P. Y., Chuang, S. E., Yeh, K. H., Yang, C. H., Kuo, M. L., and Cheng, A. L. Glucocorticoids Affect Growth and Chemosensitivity of Carcinoma Cells via Diverse Glucocorticoid Receptor-related Pathways. Proc. American Association for Cancer Res. 42: 2951, 2001.
43. Wang, C. Y., Mayo, M. W., and Baldwin, A. S. TNF-alpha and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. Science, 274: 784-787, 1996.
44. Kang, H. Y., Yeh, S., Fujimoto, N., and Chang, C. " Cloning and characterization of human prostate coactivator ARA54, a novel protein that associates with the androgen receptor". J. Biol. Chem., 274: 8570-8576, 1999.
45. Kino, T., Gragerov, A., Kopp, J. B., Stauber, R. H., Pavlakis, G. N., and Chrousos, G. P. The HIV-1 virion-associated protein vpr is a coactivator of the human glucocorticoid receptor. J. Exp. Med., 189: 51-62, 1999.
46. McKenna, N. J., Lanz, R. B., and O'malley, B. W. Nuclear receptor coregulators: cellular and molecular biology. Endocr. Reviews, 20: 321-344, 1999.
47. Weijtens, O., Schoemaker, R. C., Cohen, A. F., Romijn, F. P., Lentjes, E. G., and Van Rooij, J. Dexamethasone concentration in vitreous and serum after oral administration. Am. J. Ophthalmol., 125: 673-679, 1998.
48. Brady, M. E., Sartiano, G. P., Rosenblum, S. L., Zaglama, N. E., and Bauguess, C. T. The pharmacokinetics of single high doses of dexamethasone. Eur. J. Clin. Pharmacol., 32: 593-596, 1987.