行政院國家科學委員會補助專題研究計畫成果報告

皮質類固醇及非類固醇抗發炎劑對癌細胞藥物 敏感性的影響及相關機轉之研究

計畫類別: 個別型計畫 整合型計畫

計畫編號:NSC NSC90-2314-B-002-247

執行期間: 88 年 07 月 01 日至 91 年 06 月 30 日

- 計畫主持人: 鄭安理 國立台灣大學醫學院 內科
- 共同主持人: 郭明良 國立台灣大學醫學院 毒理所

盧彥伸 台大醫學 腫瘤科

本成果報告包括以下應繳交之附件:

赴國外出差或研習心得報告一份

赴大陸地區出差或研習心得報告一份

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

執行單位:國立台灣大學醫學院 內科部

中華民國 91年 10 月 25 日

行政院國家科學委員會專題研究計畫成果報告 皮質類固醇及非類固醇抗發炎劑對癌細胞藥物 敏感性的影響及相關機轉之研究

Study on the effects and mechanisms of action of glucocorticoids and non-steroidal anti-inflammatory drugs on the drug sensitivity of common solid tumors

計畫編號:NSC90-2314-B-002-247

執行期限: 88 年 07 月 01 日至 91 年 06 月 30 日

主持人:鄭安理 國立台灣大學醫學院 內科

共同主持人:郭明良 國立台灣大學醫學院 毒理所

盧彥伸 國立台灣大學醫學院附設醫院 腫瘤科

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。雖然類固醇己被証實 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。

在此研究中,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。Dexamethason(DEX)被選為皮質類 固醇代表藥物。我們發現:

- 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。DEX 抑制四株細 胞的生長(MCF-7, MCF/MXR1, MCF/TPT₃₀₀及 HeLa 細胞),提高了一 株細胞對 cisplatin 的化學藥物感受性 (SiHa),並降低兩株細胞對 cisplatin 的 化學藥物感受性(H460 及 Hep3B)。
- 2. 此影響是皮質類固醇受體—依賴性的。因為 DEX 有影響的七株細胞的皮質類固醇受體濃度比其他七株不受DEX 影響的細胞高許多(5.2 £.5 x10⁴ vs 1.3 ±1.4 x10⁴, P=0.005)。
- 3. 造成這些 DEX 異質性影響的相關機轉

也相同地是異質性而且彼此互斥。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。對於 MCF-7, MCF/MXR1 及 MCF/TPT₃₀₀ 細胞造成的生長抑制效應 與 G1 細胞週期停止及 p21 蛋白上升調 控有關。而 DEX 在 H460 細胞造成的 化學藥物抗藥性則與 Bcl-2 蛋白上升調 控有關。

- 在 SiHa, H460 及 Hep3B 等三株會因為 DEX 處理而改變化學藥物敏感性的細胞株中,多重性抗藥基因 MDR1 及MRP 的表現量及活性並不受 DEX 的影響。
- 5. 在此十四株細胞中非類固醇抗發炎劑 對化學藥物敏感性沒有影響。

關鍵詞:皮質類固醇、癌細胞、化學藥物 感受性

Abstract

Background: Although high-dose of glucocorticoids (GCs) are commonly co-administered with anti-cancer drugs to prevent drug-induced allergic reaction, nausea, and vomiting, little is known regarding the effects of GC on the growth or chemosensitivity of common human carcinomas. *Methods:* Ten carcinoma cell lines were randomly selected to assess the effects of GC on chemosensitivity. Cellular GC receptor (GCR) content was determined

by using ³H-labelled dexamethasone (DEX). Growth inhibition was estimated by MTT assay. Cell cycle phases and apoptosis were analyzed by flow cytometry. DNA-binding activity of NF-*k*B was measured by electrophoretic mobility shift assay (EMSA). The expression of p53, p21, p27, cyclin D1 and Bcl-2 family was determined by Western blot. Results : DEX had effects on either growth or chemosensitivity 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 decreased one (SiHa). and cisplatin cytotoxicity of 2 (H460 and Hep3B) cells lines. 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 $\pm 2.5 \times 10^4$ vs 1.3 $\pm 1.4 \times 10^4$, P=0.005) suggesting GCR is the pivotal mediator of the effect of DEX on carcinoma cell. Further, transfection of a vector encoding GCR to a GCR low-expressing cell line (AGS) increased its susceptibility to the effect of DEX. The possible mediators of the heterogeneous effects of DEX on these carcinoma cells were screened. The growth inhibitory effect of DEX was found associated with cell cycle phase regulation. DEX induced p21 up-regulation and G1 arrest in MCF-7, MCF-7/MXR1, and MCF-7/TPT300; and caused G2/M arrest in HeLa cells. The cell cycle phases of all other cell lines were not affected by DEX. The cytotoxicity-enhancing effect of DEX on SiHa cells correlated well with its effect on NF-ĸB activity: DEX suppressed cisplatin-induced activation $NF-\kappa B$ exclusively in SiHa cells, and expression of a dominant-negative truncated IkBa gene completely abolish this effect of DEX. The NF- κ B activity of all other cell lines was either not affected by cisplatin or the cisplatin-activated NF-kB not suppressible by DEX. Finally, DEX up-regulated Bcl-2 exclusively in H460, one of the two cell lines in which DEX induced cisplatin resistance. Conclusions : GCs affect growth or cisplatin chemosensitivity in some carcinoma cells. Whether this in-vitro observation is of

clinical relevance and the molecular mechanisms mediating the heterogeneous GCR-dependent effect of GC on carcinoma cells need to be clarified.

Keywords: Glucocorticoids, Carcinoma cell, Chemosensitivity

二、緣由與目的

Glucocorticoids (GC) are commonly co-administered with anti-cancer drugs in non-hematological patients with malignancies for the prevention of drug-induced allergic reaction or nausea/vomiting. Non-steroidal anti-inflammatory drugs (NSAIDs) are also widely used in the treatment of cancer pain. Although GC and NSAIDs are known to affect pertinent cellular signal transduction pathyways which are related to carcinogenesis and drug resistance, little is known regarding the possible influence of these drugs on the chemosensitivity of cancer cells.

GC and NSAIDs share several common biologic effects on the cells. They both have anti-inflammatory effect and can inhibit the activity of important cytokines such as IL-6. GC and NSAIDs are recently found to inhibit NF-kB .. Further, GC and NSAIDs may be involved in the regulation of Bcl-x, and thereby affect the apoptosis of cancer cells. **NSAIDs** also inhibit cyclooxygenase(COX) - I and COX -II, which are closely related to carcinogenesis and drug resistance. Since the GC-regulated and NSAIDs - regulated genes and mediators, including IL-6, NF-kB ,Bcl-2 family, and COX-I,II, are all known factors related to drug sensitivity of cancers, it is mandantory for us to examine there role in the chemosensitizing effect of DEX on SiHa cells.

The specific aims of the study are: (1) To systematically examine the influence of GC and NSAIDs on the drug susceptibility of common solid tumor cell lines. (2) To clarify the possible downstream mechanisms

Since GC is so commonly co-administered with anti-cancer drugs such as cisplatin and taxanes, and NSAIDs are widely used in the treatment of cancer pain, results of this study should have significant impact on the daily practice of clinical oncology.

三、結果

DEX Affected Either Growth or Chemosensitivity in 8 (MCF-7, MCF-7/MXR1, MCF-7/TPT300, HeLa, SiHa, H460, and Hep3B) of the 14 Cell Lines Tested

DEX $(0.01-1.0\mu M)$ inhibited cell growth in MCF-7, MCF-7/MXR1, MCF-7/TPT300, and HeLa cells. However, DEX alone, up to 20 µM, was not toxic to the other 10 cell lines, including AGS, N87, SNU1, SiHa, Caski, Hep3B, Hut 7, TW01, TW04, and H460. To further study the effect of GC on the chemosensitivity of carcinoma cells toward anti-cancer agents, we focused on the 10 cell lines for which GC did not cause growth suppression. Pretreatment of SiHa cells with 1 µM DEX for 24 hours decreased the IC₅₀ of cisplatin from 18.6 \pm 9.7 1.9 μМ to \pm 2.0 μM. This cytotoxicity-enhancing effect could be observed even when the concentration of DEX was as low as 1 nM or even cotreatment of DEX with cisplatin. In DEX slightly decreased contrast, chemosensitivity toward cisplatin in H460 and Hep3B cells. DEX had no effect on the chemosensitivity of AGS, N87, SNU1, Hut-7, Caski, NPC-TW01, and NPC-TW04 cells toward cisplatin.

Correlation of GCR Content with GC Effect

The GCR contents of these 14 cell lines are measured. 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 ($5.2 \pm 2.5 \times 104 \text{ vs.} 1.3 \pm 1.4 \times 104$, P=0.005) suggesting GCR is the pivotal mediator of the effect of DEX on carcinoma cell. The GCR content of human lymphocytes, the internal control for these experiments, was within the reported range (2,500 ~ 5,400 sites/cell) (17).

DEX has no effect on the NPC-TW01 and NPC-TW04 cells despite of high GCR content (4.2 $\times 10^4$ and 2.0 $\times 10^4$, respectively) noted in these two cell lines. Therefore, we

examined the function of the GCR in these two cell lines by MMTV-Luc reporter assay. In MCF-7 cells transfected with MMTV treatment with DEX reporter plasmid, induced luciferase activity. However, in NPC-TW01 and NPC-TW04 cells transfected with MMTV reporter plasmid, treatment with DEX dose not induced luciferase activity. On the other hand, when the NPC-TW01 and NPC-TW04 cells were co-transfection with MMTV reporter plasmid and pS-GCR, which contains human GCR gene, treatment with DEX could induce luciferase activity in these two cell lines. These data showed that the function of GCR, at least in transcription activity on GRE, in NPC-TW01and NPC-TW04 cells is impaired.

Transfection of GCR in AGS Cells Increased its Susceptibility to the Effect of DEX

To further examine whether the GCR content is pivotal in mediating the susceptibility to DEX in carcinoma cell, we transfected pS-GCR for increasing the GCR content to AGS, a GCR low-expressing cell line, The expression of the total GR amount in vehicle transfected AGS cells, pooled stably pS-GCR transfected AGS cells, and single cell cloned pS-GCR transfected AGS cells were examined by $[^{3}H]$ DEX binding assay. The treatment of DEX alone has no effect on the growth of pS-GCR transfected AGS cells as examined by the MTT assay. However, pS-GCR transfected AGS cells that expressing high GCR content became susceptible to the effect of DEX on chemoresistance toward cisplatin.

DEX Caused Cell Cycle Arrest only in Growth-suppressible Cells

Among the 14 cell lines tested, DEX caused cell cycle G1 phase arrest in MCF-7, MCF-7/MXR1, MCF-7/TPT300 cells, and G2/M phase arrest in HeLa cells. To examine the possible mechanism of DEX-induced G1 arrest in these cell lines, the protein level of cell cycle related protein, including p21, p27, cyclin D1 and p53, was analyzed by Western blotting. In MCF-7, MCF-7/MXR1, and MCF-7/TPT300 cells, the p21 level increased after DEX treatment. There was no change

of p21 level after DEX treatment in the other 7 cell lines. The level of p27, cyclin D1, and p53 were not affected by DEX treatment in all cell lines tested.

DEX Suppressed Cisplatin-induced NF-κB Activation Exclusively in SiHa Cells

To explore the mechanism underlying the chemosensitizing effect of DEX in SiHa cells, EMSA assay of NF-kB DNA binding activity and reporter luciferase assay of NF- κ B transcription activity were done. NF- κ B DNA binding activity transiently increased after exposure to 20 μ M (IC₅₀) cisplatin. This NF- κ B DNA binding activity was blocked by pre-incubating the cells with 1 µM DEX. The transactivating activity of NF- κ B on its cis elements was further verified in SiHa cells stably transfected by a reporter construct containing five NF- κ B binding sites. Treatment with cisplatin (20~200 µM) resulted in the induction of luciferase activity, which could be repressed by pretreament with DEX. The effect of DEX on the IkB expression in SiHa cells was also examined. Western blot analysis of whole-cell protein showed that DEX did not upregulate the expression of IkB in SiHa cells. By contrast, in other cell lines, NF- κ B was either not activated by TNF- α or (H460), cisplatin-activated cisplatin or NF-*κ*B was not suppressible by DEX (Hep3B, AGS, SNU1, and N87). These data suggest that protein-protein interaction between activated GCR and NF- κ B plays a central role in the chemosensitizing effect of DEX in SiHa cells.

To further examine the role of NF- κ B in the cytotoxicity-enhancing effect of DEX, we generated a re-combinant plasmid containing dominant negative I κ B α (dnI κ B α) gene. This dnI κ B α protein does not contain the residues necessary for signal-induced phosphorylation and proteasome-mediated degradation of I κ B α , thereby preventing dissociation and translocation of NF- κ B to the nucleus. The expression of the dnI κ B α in pooled stably transfected SiHa cells was verified by Western blot analysis. the control-pRCMVtransfected SiHa cells contained only the

endogenous wild-type I κ B α protein, while the dnIkBa-pRCMV-transfected SiHa cells contained an additional band representing the truncated exogenous IkBa protein. Results of EMSA showed that NF- κ B binding activity was markedly suppressed in the dnIkBa-pRCMV-transfected cells after either TNF- α or cisplatin treatment. In addition, the cytotoxicity-enhancing effect of DEX in dnIkBa-pRCMV-transfected SiHa cells was abolished. The dnIkBa-pRCMV-transfected SiHa cells had also become more sensitive to cisplatin as compared to the control-pRCMV-transfected cells. SiHa These data confirmed that NF- κ B plays a central role in the chemosensitizing effect of DEX in SiHa cells.

DEX Up-regulated Bcl-2 Expression Exclusively in H460 Cells

To explore the mechanisms of DEX-induced cisplatin resistance in H460 and Hep3B cells, the expression of Bcl-2, Bax, and Bcl- $X_{L/S}$ was examined by Western blot analysis. In H460 cells, Bcl-2 protein was up-regulated by DEX treatment; while BAX, and Bcl- $X_{L/S}$ were not affected. In contrast, none of these protein levels was affected by DEX in Hep3B cells.

NSAIDs have no effect on the chemosensitivity on these cells.

effect of **NSAIDs** the The on chemosensitivity on these cell lines has also tested. Aspirin, indomethasin, been piroxicam were selected as representative NSAIDs. The maximum non-toxic dose of these NSAIDs were selected to pretreat these 15 carcinoma cell lines. Then the effect on the chemosensitivity of taxol, cisplatin, 5-FU, doxorubicin were tested by the MTT test. We found that NSAIDs has no effect on the chemosensitivity on these cells.

四、討論

This study has clearly demonstrated that GC affects either growth or chemosensitivity in a substantial portion of carcinoma cells. Since GC are so commonly co-administered with anticancer drugs such as taxanes and platinums, these effects of GC may have to be taken into consideration in the treatment

of cancer patients. It may not be difficult to identify those carcinoma patients who are going to be affected by GC, since, as demonstrated in this study, only cells with high GCR content are affected. However, how GC will affect these cancer patients remains unknown. As demonstrated in this study, the effects of GC on various carcinomas are extremely diverse. It is therefore mandatory to identify the cellular mechanisms that dictate the ultimate effect of GC.

A direct correlation between GCR content and the magnitude of the physiologic response to GC has been reported (1). The sensitivity of many lymphoid cell lines to GC-induced growth arrest and apoptosis is directly proportional to intracellular receptor content (2-4).This study clearly demonstrated that the susceptibility to the effect of DEX on cell growth or chemosensitivity in carcinoma cells are also correlate well with the level of GCR content. However, the GCR contents of the carcinoma cells on which GC has an effect is almost 10 times higher than that of lymphoid cells (2-4), which are usually supersensitive to GC, suggesting that the cellular contexts and the signal transduction pathways for GC-GCR are different between these two groups of cells.

In this study, we found that DEX growth MCF-7. inhibits cell in MCF-7/MXR1, MCF-7/TPT300, and HeLa cells. Parallel to this growth inhibition, DEX causes G1 arrest in the MCF-7. MCF-7/MXR1, MCF-7/TPT300 cell lines, and G2/M arrest in HeLa cells. DEX had no effect on the regulation of the cell cycle in the 10 cell lines on which DEX had no growth inhibitory effect. It has recently been shown that in some cell types, the level of p21 increases in response to GCR activation (5, 6). Naumann et al. reported a p53-independent p21 accumulation after GC treatment in a glioma cell line (7). A GC responsive region in the p21 promoter has been localized to a binding site for CCAT/enhancer-binding protein α (C/EBP α) (8, 9), expression of which is GCR inducible

(10). In this study, DEX induced p21 expression exclusively in MCF-7, MCF-7/MXR1, and MCF-7/TPT300 cells. This may partly explain the mechanism of DEX-induced G1 arrest in these cells. However, we do not observe the expression of p21 induced by DEX in HeLa cells, suggesting the diversified effects of GC is cell-context dependent.

GCR exerts its cellular effect by two distinctive pathways, either transactivation of specific genes or direct protein-protein interaction with other transcriptional factors. Upon binding to its ligands, cytoplasmic GCR enters the nucleus, dimerizes, binds to specific DNA sequences called glucocorticoid response elements (GREs), and transactivates target genes, such as those for tyrosine aminotransferase and alanine aminotransferase (11). However, some major effects of GC, including anti-inflammatory and immunosuppressive effects, are achieved by regulating genes that do not contain GREs in their promoters (12, 13, 14). The suppression of NF- κ B activity has been suggested to be the major mechanism of the anti-inflammatory effect of GC (15-18). Activated GCR physically interact with Rel A, the subunit of NF- κ B, and thereby suppress the activation of NF- κ B (15-18). Activation of NF- κ B has been implicated in mediating drug resistance of cancer cells. NF- κ B could be activated by a variety of stresses, including oxidative stress and DNA damage (19-23). Activated NF-*k*B may prevent the triggering of apoptosis, and thus drug result in resistance against DNA-damaging agents (24-27).The molecular mechanism of NF-kB-mediated protection of cells remains unclear, but may involve the up-regulation of caspase inhibitors (28). In this study, we have provided evidence that NF- κ B plays an important role in mediating the drug resistance of SiHa cells. Suppression of NF- κ B activity by dnI κ B α 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- κ B.

As expected, we do not observe the similar phenomena on H460 and Hep3B cells, on which DEX induced a drug resistance. It remains to be clarified why certain carcinoma cell lines do not have an NF- κ B response to cisplatin or have an NF- κ B response which cannot be attenuated by DEX. DEX has been reported to suppress drug-induced apoptosis in a human gastric cancer cell line, probably through regulation of bcl-2 gene family (29, 30). The products of the bcl-2 gene family regulate pertinent cellular pathways for apoptosis in response to a variety of stimuli Our results also showed (31). that DEX-mediated up-regulation of Bcl-2 were found exclusively in H460 cells, suggesting that the chemoresistance-inducing effect of GC was partly mediated via regulation of the Bcl-2 family. However, the mechanism of the chemoresistance-inducing effect of GC in Hep3B cells remains elusive.

Multidrug exporter proteins may have played a role in mediating GC-induced drug resistance. Donald et al. reported that GCs including DEX are capable of inhibiting the function of P-glycoprotein of mouse thymoma cell line and human lymphoblastic leukemic cell lines (51). On the other hand, Anthony et al. reported that DEX up-regulates the activity of P-glycoprotein of an immortalized rat brain endothelial cell line (33). GC has also been shown to regulate the expression of MDR-1 gene (34, 35). Although cisplatin is not a substrate of the multidrug exporter proteins, we have screened the effect of DEX on the expression and function of ABC, and we found no evidence that MDR-1, MRP or other multidrug exporters have played a role in mediating GC-induced change of drug sensitivity.

Our data have shown that GC exerts multi-functional effects on different types of cells and involved different phases of cell cvcle control. The effects and the downstream mechanisms GC of on carcinoma cells are diverse. Although high-GCR content is necessary in the response to GC, the diversified effects of GC cannot be explained simply by the amount of

GCR. These 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 the possible explanation for the diverse GC effects. Co-regulators of AR may play 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 be androgenic (36, 37). Several novel co-regulators of GC have also been found to play important roles in the signaling pathway of the GCR (38). Clarification of whether co-regulators of GCR play a role in segregating the direction of downstream pathways in GC-treated carcinoma cells is mandatory.

After a single oral dose of 7.5 mg DEX, the serum concentration of DEX was found to be around 0.12 μ M for 1~3 hours (39). However, the serum concentration of DEX may reach 2 µM after a single intravenous infusion of 80~100 mg (40). Since the administration of high dose DEX is widely used for the prevention of cisplatin-induced nausea/vomiting, the possible effect of GC on the chemosensitivity of some selected cancers needs to be seriously considered. In summary, the results of this study suggest that GC exerts a GCR-dependent effect on growth or chemosensitivity in a the substantial portion of carcinoma cells. The clinical relevance and the cellular mechanisms that dictate the disparate effects of GC need to be further clarified.

六、參考文獻

(1) Vanderbilt JN, Miesfeld R, Maler BA, Yamamoto KR. Intracellular receptor concentration limits glucocorticoiddependent enhancer activity. Mol Endocrinol 1987;1:68-74.

(2) Gehring U, Mugele K, Ulrich J. Cellular receptor levels and glucocorticoid responsiveness of lymphoma cells. Mol Cell Endocrinol 1984;36:107-13.

(3) Bourgeois S, Newby RF. Diploid and haploid states of the glucocorticoid receptor gene of mouse lymphoid cell lines. Cell 1977;11:423-30.

(4) Chapman MS, Askew DJ, Kuscuoglu U, Miesfelt RL. Transcritional control of steroid-regulated apoptosis in murine thymoma cells. Mol Endocrinol 1996;10:967-78.

(5).. Ramalingam A, Hirai A, Thompson EA. Glucocorticoid inhibition of fibroblast proliferation and regulation of the cyclin kinase inhibitor p21Cip1. Mol Endocrinol 1997;11:577-86.

(6) Rogastsky I, Trowbridge JM, Garabedian MJ. Glucocorticoid receptor-mediated cell cycle arrest is achieved through distinct cell-specific transcriptional regulatory mechanisms. Mol Cell Biol 1997;17:3181-93.

(7) Naumann U, Durka S, Weller M.
Dexamethasone-mediated protection from drug cytotoxicity: association with p21
WAF1/CIP1 protein accumulation?
Oncogene 1998;17:1567-75.

(8) Burnstein KL, Jewell CM, Sar M, Cidlowski JA. Intragenic sequences of the glucocorticoid human receptor complementary DNA mediate hormone-inducible receptor messenger RNA down-regulation through multiple mechanisms. Mol Endocrinol 1994;8:1764-73.

(9) Cha HH, Cram EJ, Wang EC, Huang AJ, Kasler HG, Firestone GL. Glucocorticoids stimulate p21 gene expression by targeting multiple transcriptional elements within a steroid responsive region of the p21waf1/cip1 promoter in rat hepatoma cells. J Biol Chem 1998;73:1998-2007.

(10) Ramos RA, Nishio Y, Maiyar AC, Simon KE, Ridder CC, Ge Y, et al. Glucocorticoid-stimulated

CCAAT/enhancer-binding protein α expression is required for steroid-induced G1 cell cycle arrest of minimal-deviation rat hepatoma cells. Mol Cell Biol 1996;16:5288-301

(11) Beato M, Herrich P, Schutze G. Steroid hormone receptors: many actors in search of a plot. Cell 1995;83:851-7

(12) McEwan IJ, Wright AP, Gustafsson JA. Mechanism of gene expression by the glucocorticoid receptor: role of protein-protein interactions. Bioessays 1997;19:153-60.

(13) Cato AC, Wade E. Molecular mechanisms of anti-inflammatory action of glucocorticoids. Bioessays 1996;18:317-78.

(14). Van der Burg B, Okret S, Liden J, Wissink S, Van der Saag PT, Gustafsson JA. Nuclear factor-kappa-B repression in anti-inflammation and immunosuppression by glucocorticoids. Trends Endocrinol Metab 1997;8:152-7.

(15) Ray A, Prefontaine KE. Physical association and functional antagonism between the p65 subunit of transcription factor NF-kB and the glucocorticoid receptor. Proc Natl Acad Sci USA 1994;91:752-6.

(16) Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin AS. Characterization of mechanisms involved in transrepression of NF-kB by activated glucocorticoid receptors. Mol Cell Biol 1995;15:943-53.

(17) Caldenhoven E, Liden J, Wissink S, Van der Stolpe A, Raaijmarkers J, Koenderman L, et al. Negative crosstalk between RelA and the glucocorticoid receptor: a possible mechanism for the anti-inflammatory action of glucocorticoids. Mol Endocrinol 1995;9:401-12.

(18) Wissink S, van Heerde EC, Schmitz ML, Kalkhoven E, van der Burg B, Baeuerle PA, et al. Distinct domains of the Rel A NF-kB subunit are required for negative cross-talk an direct interaction with the glucocorticoid receptor. J Biol Chem 1997;272:22278-84.

(19) Sen, C. K. and Packer, L. Antioxidant and redox regulation of gene transcription. FASEB J., 10: 709-720, 1996.

(20) Friedberg, E. C., Walker, G. C., and Siede, W. DNA repair and mutagenesis. pp. 14-19. ASM Press: Washington D.C., 1995.

(21) Bertoncini, C. R. and Meneghini, R. DNA strand breaks produced by oxidative stress in mammalian cells exhibit 3'-phosphoglycolate termini. Nucleic Acids Res. 23: 2995-3002, 1995.

(22) Piret, B. and Piette, J. Topoisomerase poisons activate the transcription factor NF-kappaB in ACH-2 and CEM cells. Nucleic Acids Res. 24: 4242-4248, 1996.

(23) Quinto, I., Ruocco, M. R., Baldassarre, F., Mallardo, M., Dragonetti, E., and Scala, G.

The human immunodeficiency virus type 1 long terminal repeat is activated by monofunctional and bifunctional DNA alkylating agents in human lymphocytes. J. Biol. Chem. 268: 26719-26724, 1993.

(24) 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.

(25) Beg, A. A. and Baltimore, D. An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. Science 274: 782-784, 1996.

(26) Barinaga M. Life-death balance within the cell. Science, 274: 724, 1996.

(27) Wang, C. Y., Mayo, M. W., and Baldwin, A.. S. TNF-alpha and cancer therapy-induced apoptosis: potentiation by inhibition of NF- κ B. Science, 274: 784-787, 1996.

(28) Chu, Z. L., McKinsey, T. A., Liu, L., Gentry, J. J., Malim, M. H., and Ballard, D.
W. 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, 1997.

(29) Chang, T. C., Hung, M. W., Jiang, S. Y., Chu, J. T., Chu, L. L., and Tsai, L. C. Dexamethasone suppresses apoptosis in a human gastric cancer cell line through modulation of bcl-x gene expression. FEBS Lett., *415*: 11-15, 1997.

(30) Chang, T. C., Tsai, L. C., Hung, M. W., Chu, L. L., Chu, J. T., and Chen, Y. C. Effects of transcription and translation inhibitors on a human gastric carcinoma cell line. Potential role of Bcl-X(S) in apoptosis triggered by these inhibitors. Biochem. Pharmacol., 53: 969-977, 1997.

(31) Vaux, D. L. and Strasser, A. The molecular biology of apoptosis. Proc. Natl. Acad. Sci. USA, 93: 2239-2244, 1996.

(32) Gruol, D. J. and Bourgeois, S. Chemosensitizing steroids: glucocorticoid receptor agonists capable of inhibiting P-glycoprotein function. Cancer Res., 57: 720-727, 1997.

(33) Regina A, Romero IA, Greenwood J, Adamson P, Bourre JM, Couraud PO, et al.

Dexamethasone regulation of P-glycoprotein activity in an immortalized rat brain endothelial cell line, GPNT. J Neurochem 1999;73:1954-63.

(34) Demeule M, Jodoin J, Beaulieu E, Brossard M, Beliveau R. Dexamethasone modulation of multidrug transporters in normal tissues. FEBS Letters 1999;42:208-14.

(35) Zhao JY. Ikeguchi M. Eckersberg T. Kuo MT. Modulation of multidrug resistance gene expression by dexamethasone in cultured hepatoma cells. Endocrinol 1993;133:521-8.

(36) Kang HY, Yeh S, Fujimoto N, Chang C. Cloning and characterization of human prostate coactivator ARA54, a novel protein that associates with the androgen receptor. J Biol Chem 1999;274:8570-6.

(37) Kino T, Gragerov A, Kopp JB, Stauber RH, Pavlakis GN, Chrousos GP. The HIV-1 virion-associated protein vpr is a coactivator of the human glucocorticoid receptor. J Exp Med 1999;189:51-62.

(38) McKenna NJ, Lanz RB, O'malley BW. Nuclear receptor coregulators: cellular and molecular biology. Endocr Reviews 1999;20:321-44.

(39) Weijtens O, Schoemaker RC, Cohen AF, Romijn FP, Lentjes EG, Van Rooij J, et al. Dexamethasone concentration in vitreous and serum after oral administration. Am J Ophthalmol 1998;125:673-9.

(40) Brady ME, Sartiano GP, Rosenblum SL, Zaglama NE, Bauguess CT. The pharmacokinetics of single high doses of dexamethasone. Eur J Clin Pharmacol 1987;32:593-6.