J. Steroid Biochem. Molec. Biol. Vol. 38, No. 5, pp. 593–598, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

FUNCTIONAL ABNORMALITY OF GLUCOCORTICOID RECEPTOR IN SHIONOGI CARCINOMA 115 CELLS AS EVIDENCED BY GENE TRANSFER EXPERIMENTS

TAKAHISA HIROSE,[1] MASAFUMI KOGA,[1] HIROSHI SAITO,[1] HARUHIKO KOUHARA,[1] SATORU SUMITANI,[1] SOJI KASAYAMA,[1] KEISHI MATSUMOTO[2] and BUNZO SATO[1]*

[1]The Third Department of Internal Medicine and [2]The Second Department of Pathology, Osaka University Hospital, Fukushima-ku, 1-1-50, Osaka 553, Japan

(Received 2 August 1990)

Summary—The assay systems for steroid receptor functions in steroid-sensitive cells (SC-3 cells) were developed in which hormone-responsive element linked to a reporter gene [chloramphenicol acetyl transferase (CAT) gene] was transfected by the electroporation technique. Stimulation with androgen of SC-3 cells transfected with mouse mammary tumor virus promoter-CAT gene (MMTV-CAT) resulted in clear enhancement of CAT activity, whereas glucocorticoid required abnormally high concentrations to obtain significant stimulation. The simultaneous addition of glucocorticoid surprisingly inhibited androgen-induced CAT activity in SC-3 cells, whereas glucocorticoid and androgen acted together synergistically to activate CAT activity in T 47D cells. When SC-3 cells were cotransfected with the expression vector of human glucocorticoid receptor (GR) gene, inhibition with glucocorticoid of androgen-enhanced CAT activity was abolished. These results would suggest that SC-3 cells contain functionally abnormal GR.

INTRODUCTION

The steroid hormone action mechanism has been extensively studied, especially in relation to cancer cell growth [1]. Since the presence of functional steroid receptors is an essential component in steroid-induced growth modulation, many studies have been directed toward characterizing steroid receptors. Indeed, most of the steroid-resistant clones isolated from mouse and human transformed lymphoid cells have been shown to be defective in some aspect of glucocorticoid receptor (GR) function [2–5]. The vast majority of resistant cells isolated from mouse cells after chemical mutagenesis displayed little or no steroid binding activity [6, 7]. Thus, the GR gene appears to be the primary target for mutations which result in glucocorticoid resistance. These abnormalities in steroid receptors have been mainly identified by analyzing their ligand binding abilities and hydrodynamic parameters. The presence of mutated steroid receptors with normal ligand binding ability can be expected. In this relation, it has been well recognized that only 60% of estrogen receptor(ER)-positive breast cancers could respond to hormone therapies [8].

Recent experimental results have established that many *cis*-acting elements as well as *trans*-acting factors other than steroid receptors are involved in the process of steroid hormone-dependent gene activation [9–11]. For instance, progression to steroid insensitivity has been observed to occur even in cells containing functional steroid receptors [12]. Therefore, the differentiation of receptor abnormality from postreceptor abnormality is required to examine abnormal regulation of gene activation with steroid hormones.

The mouse mammary carcinoma, Shionogi carcinoma 115 (SC-115), is one of the most extensively characterized steroid hormone-dependent tumors. The growth of SC-115 has been reported to be stimulated *in vivo* by physiological concentrations of androgen and by nonphysiological high doses of glucocorticoid or estrogen *in vivo* [13–15]. SC-3 cells cloned from SC-115 have been observed to be growth-stimulated by androgen or glucocorticoid in the serum-free culture condition [14, 16]. To obtain the maximum growth-stimulatory effects on SC-3 cells, a relatively high concentration of

*To whom all correspondence should be addressed.

glucocorticoid [10^{-6} M dexamethasone (Dex)] is again required, while 10^{-8} M testosterone (T) is enough for elucidating the maximum growth-response. Moreover, the growth-stimulatory ability of T is much higher than that of Dex. This quantitative difference between androgen- and glucocorticoid-dependent growth of SC-3 cells cannot be attributed to the difference in genes activated by these steroids, since the growth-stimulatory ability of both steroids is antagonized by the antibody against basic fibroblast growth factor [17]. This consideration might be supported by our previous observation that both steroids stimulate the synthesis and secretion of 24 K protein in SC-3 cells [18]. Interestingly, glucocorticoid has been observed to antagonize androgen-dependent enhancement of SC-3 cell growth [19]. The molecular basis for these positive and negative effects of glucocorticoid remains to be elucidated. To clarify these unresolved problems, gene transfer experiments were carried out using the reporter plasmid as well as authentic GR gene.

MATERIALS AND METHODS

Chemicals

Eagle's minimum essential medium (MEM), Dulbecco's modified Eagle medium (DMEM) and Ham's F-12 were obtained from Nissui Pharmaceutical Co. Ltd (Tokyo, Japan). Fetal calf serum (FCS) was from Irvine Scientific (Santa Ana, Calif., U.S.A.). D-Threo-[dichloroacetyl-1,2-^{14}C]chloramphenicol (60 mCi/mmol) (CAT assay grade) was from New England Nuclear (Cambridge, Mass., U.S.A.). The restriction enzymes were purchased from Toyobo Co. Ltd (Tokyo, Japan). T, Dex, bovine serum albumin (BSA) (essential fatty acid free) and Norit A (acid-washed charcoal) were from Sigma Chemical Co. (St Louis, Mo., U.S.A.). The other chemicals used here were of analytical grade. The expression vector of human GR was kindly provided by Dr R. M. Evans [20]. Plasmid pCH 110 (coding for β-galactosidase activity) and MMTV-CAT (pMSG-CAT) were purchased from Pharmacia (Uppsala, Sweden).

Cell culture and cell growth experiment

SC-3 cells from SC-115 tumor were cloned and maintained in MEM supplemented with 10^{-8} M T and 2% dextran-coated charcoal(DCC)-treated FCS, as described previously [15]. To examine the growth-stimulatory effects of steroids, SC-3 cells were plated onto three replicate 35-mm dishes (2×10^{-4} cell/dish) containing 2 ml MEM containing 2% DCC-treated FCS (no hormone). On the following day (day 1), the medium was changed to 2 ml serum-free medium [Ham's F-12:MEM (1:1, v/v) containing 0.1% BSA] with various steroids. The serum-free medium was changed every other day, and the number of cells were counted on day 7. T 47D cells, human breast cancer cells, were maintained in DMEM containing 10% FCS.

Transfection and CAT assays

The subconfluent cells were harvested and washed with 0.272 M sucrose, 7 mM phosphate, 1 mM $MgCl_2$, pH 7.4, at 20°C (PBSM buffer). The aliquots (0.8 ml) of the cell suspension in PBSM buffer (2.7×10^7 cells/ml) were transferred into the gene pulser cuvette (Bio-Rad; Richmond, Calif., U.S.A.) containing the reporter plasmid DNA (20 μg) with or without human GR expression vector (5 μg). In some experiments, pCH 110 plasmid (10 μg) was also included. These cuvettes were twice exposed to the electroporation procedure (400–420 V, 25 μFD). Each exposure was followed by cooling at 0°C for 10 min. These cells were then plated onto 60-mm culture dishes in 5 ml of phenol red-free MEM supplemented with 10% DCC-treated FCS in the presence of various hormones. After 48 h cultures, the cells were harvested and washed twice with phosphate buffered saline (PBS). The cell pellets were resuspended in 120 μl of 0.25 M Tris HCl, pH 7.8, at 20°C and lysed by three cycles of freezing and thawing. The cell extracts were obtained by centrifugation at 10,000 *g* for 10 min. Assays for CAT activity were usually performed in a final volume of 145 μl containing 70 μg extracted cellular protein, acetyl co-enzyme A (0.5 mM) and 0.1 μCi [^{14}C]chloramphenicol [21]. This protein concentration of the cellular extracts was chosen because CAT activity was found to be linear up to 100 μg/tube. These mixtures were incubated at 37°C for 1 h. Samples were subjected to extraction and TLC. After being exposed to Kodak X-Omat AR films at −70°C for appropriate periods (1–3 days), chloramphenicol acetylation was quantified by densitometric analyses. β-Galactosidase activities were assayed as described by Herbomel *et al.* [22].

Statistical analysis

The data presented here are expressed as mean ± SE. The paired student's *t*-test is used to discuss the significant difference.

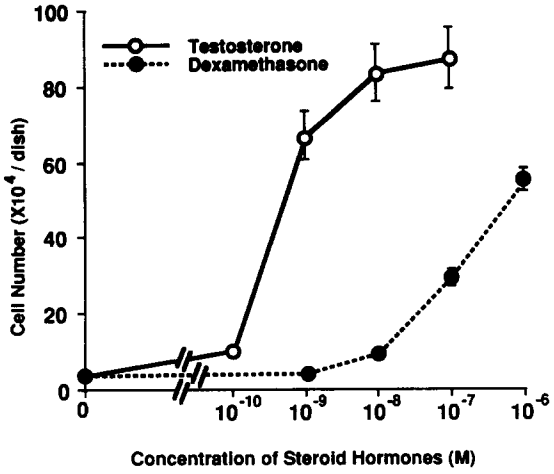

Fig. 1. Growth-stimulatory effects of T or Dex on SC-3 cells. SC-3 cells were plated and cultured in the presence of various concentrations of steroid hormones, as described in Materials and Methods. The cells were counted on day 7 by means of a Colter counter.

RESULTS

The growth-stimulatory effects of various concentrations of steroids on SC-3 cells in serum-free medium

SC-3 cells were cultured with 10^{-10}–10^{-6} M concentrations of T or Dex for 6 days in a serum-free medium and their effects on cell yield were examined (Fig. 1). T stimulation resulted in a remarkable enchancement of the cell proliferation in a concentration-dependent manner. The maximum effect of T was obtained at 10^{-8} M. With Dex, a relatively high concentration (10^{-6} M) was required to obtain the maximum effect, and its value was 62% of that induced by T. To investigate the molecular mechanism of steroid-enhanced cell growth, detailed information on their receptors was desirable. Our previous experiments on whole cell binding assays [14, 16, 19] revealed the presence of AR, GR and ER in SC-3 cells.

The ability of endogenous steroid receptors to stimulate MMTV-CAT gene transfected into SC-3 cells

To test the functional properties of steroid receptors, the reporter plasmid was transfected into SC-3 cells. The plasmid used in the present studies was the MMTV-CAT gene. After stimulation of transfected cells with hormonal steroids for 48 h, their effects on CAT gene expression were determined. β-Galactosidase activities in SC-3 cells transfected with pCH 110 as a reference plasmid were also assayed and found not to differ significantly, irrespective of hormonal stimulation (data not illustrated). The lack of a significant difference in this enzyme activity between hormone-stimulated and hormone-unstimulated cells might be due to the experimental condition where cells for CAT assay were cultured in 10% DCC-treated FCS. Hormonal stimuli for 48 h did not increase the cell yield at this FCS concentration (data not shown). Therefore, the correction for transfection efficiency and cell number difference was not made. The quantitative measurement of the MMTV-CAT gene expression was carried out by a densitometric analysis to examine the concentration-dependency of glucocorticoid and androgen. As shown in Fig. 2, T had maximum potency at 10^{-8} M, while the maximum stimulation with Dex was obtained at 10^{-6} M. The significant stimulatory effect of Dex could not be observed at 10^{-8} M. In addition, the value obtained by 10^{-6} M Dex was approx. 60% of that by 10^{-8} M T. A further increase in Dex concentration could not be performed due to its nonspecific toxic effect [19].

Inhibitory effects of Dex on androgen-induced expression of MMTV-CAT gene in SC-3 cells

We have previously reported that Dex has a stimulatory and inhibitory effect on androgen-induced SC-3 cell growth [14, 19]. Thus, the effects of Dex on androgen-dependent MMTV-CAT gene expression were investigated. As shown in Fig. 3, the physiological concentration (10^{-8} M) of Dex was found to markedly inhibit

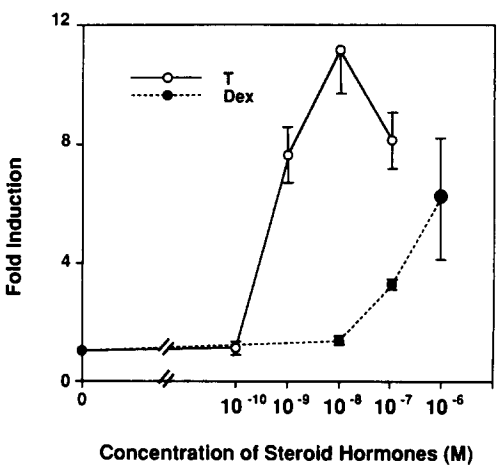

Fig. 2. Densitometric quantification of hormone effects on CAT activities in SC-3 cells transfected with MMTV-CAT plasmid. SC-3 cells transfected with MMTV-CAT plasmid were stimulated with various concentrations of T or Dex. CAT activities were quantified by densitometric scanning. The data (mean ± SE) were obtained by 3 separate experiments and expressed as fold increases over hormone-unstimulated samples.

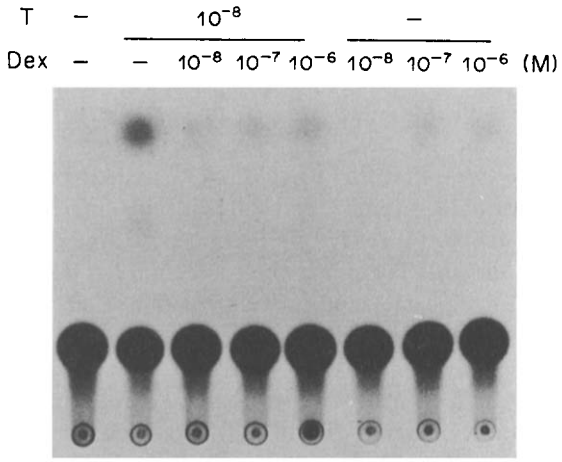

Fig. 3. Effect of Dex on T-induced CAT activities in SC-3 cells transfected with MMTV-CAT plasmid. SC-3 cells transfected with MMTV-CAT plasmid were stimulated with Dex alone or 10^{-8} M T in the presence of various concentrations of Dex. CAT assays were carried out as described in Materials and Methods.

T-stimulated MMTV-CAT gene expression. Increasing concentrations of Dex resulted in partial recovery of CAT activities. These data were quantitatively analyzed by densitometric scanning (Fig. 4). Significant inhibition of T-induced CAT activity was observed by the simultaneous addition of 10^{-8} M Dex. To exclude the possibility that the inhibitory effects of Dex on androgen-induced gene expression can be seen in all cells containing both AR and GR, MMTV-CAT plasmid was transfected into T 47D cells (Fig. 5). Both T and Dex clearly stimulated CAT gene expression at a concentration of 10^{-8} M. More importantly, the simultaneous addition of 10^{-8} M Dex did not inhibit, but stimulated, the CAT activities

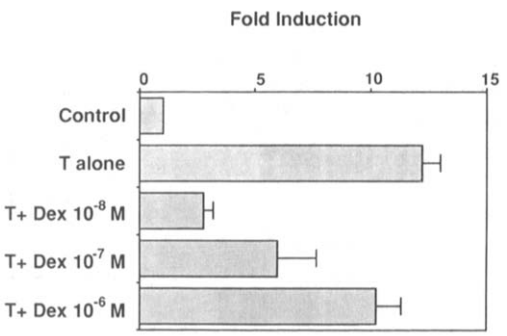

Fig. 4. Inhibitory potency of Dex on androgen-stimulated MMTV-CAT gene expression in SC-3 cells. SC-3 cells transfected with MMTV-CAT plasmid were cultured in the presence of 10^{-8} M T with various concentrations of Dex for 48 h. Then, CAT assays were done. Acetylated chloramphenicol spots were quantified by densitometric scanning, and expressed as fold increases over hormone-unstimulated samples. These values were obtained by 3 separate experiments.

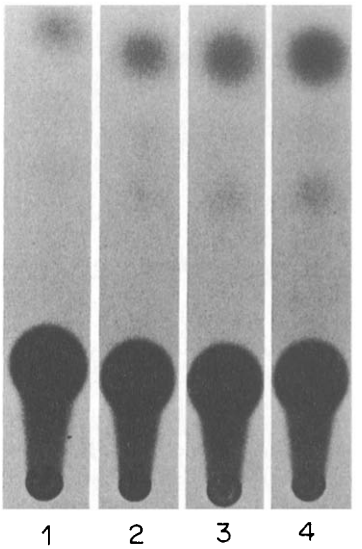

Fig. 5. Lack of inhibitory effect of Dex on MMTV-CAT gene expression in T 47D cells. T 47D cells were transfected with MMTV-CAT plasmid, followed by stimulation with 10^{-8} M T and/or 10^{-8} M Dex for 48 h. Then, CAT assays were carried out. Hormone treatments were as follows: lane 1, unstimulated; lane 2, 10^{-8} M T; lane 3, 10^{-8} M Dex; lane 4, 10^{-8} M T + 10^{-8} M Dex. The same experiment was repeated once more with a similar result.

enhanced by 10^{-8} M T. The CAT activity stimulated with both T and Dex was 1.5- to 2-fold higher than those stimulated with either Dex or T. These results would suggest

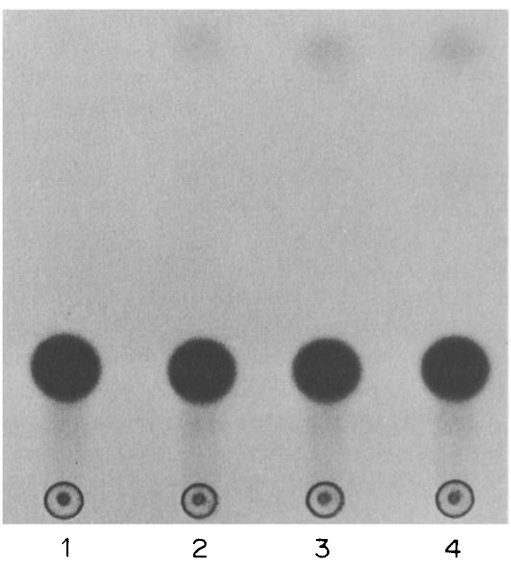

Fig. 6. Lack of negative effect of Dex on MMTV-CAT gene expression in SC-3 cells cotransfected with human GR expression vector. SC-3 cells were cotransfected with MMTV-CAT plasmid and human GR expression vector (5 μg), and unstimulated (lane 1) or stimulated with 10^{-8} M T (lane 2), 10^{-8} M Dex (lane 3) or 10^{-8} M T + 10^{-8} M Dex (lane 4) for 48 h. Then, CAT assays were carried out. The same experiment was repeated once more with a similar result.

Dex-mediated inhibition of MMTV-CAT gene expression is uniquely observed in SC-3 cells.

This interesting effect of Dex in SC-3 cells may be due to abnormal functions of GR or other *trans*-acting factors. To obtain some clue for differentiating these two possibilities, a plasmid containing human GR expression vector [20] was cotransfected into SC-3 cells. As illustrated in Fig. 6, expression of human GR resulted in a clear stimulation of CAT activities with 10^{-8} M Dex which was quantitatively similar to that obtained with 10^{-8} M T. Furthermore, an induction of human GR in SC-3 cells abolished the inhibitory potency of Dex on androgen-stimulated MMTV-CAT expression.

DISCUSSION

Functional analyses of steroid receptors using the gene transfer techniques described herein reveal that SC-3 cells contain functionally abnormal GR. The most prominent finding is that Dex inhibits androgen-dependent activation of MMTV-CAT gene. MMTV has been well recognized as containing the glucocorticoid/progestin/androgen-responsive element in the long terminal repeat (LTR) [23, 24]. Although progestin-induced transcriptional activation of MMTV-CAT has been reported to be inhibited by ER in a ligand-dependent manner [10], this seems to be the first report demonstrating that the transcriptional interference between AR and GR actually occurs on MMTV-CAT gene. This negative transcriptional effects of GR on MMTV-CAT expression seems to be limited to SC-3 cells. Furthermore, glucocorticoid-dependent stimulation of MMTV-CAT gene expression was observed when human GR expression plasmid was cotransfected into SC-3 cells. Several possible mechanisms can be considered to explain these unique events. Our previous observations have revealed that glucocorticoid treatment does not decrease AR content in SC-3 cells [19], excluding the possibility of heterologous down-regulation of AR. It can be theoretically considered that inhibition is due to the formation of nonfunctional heterodimers between the interfering GR and the activating AR.

Another possible mechanism is the competition between positive AR and less active GR for overlapping sequences. Studies on various transcriptional factors indicate that this type of competition is actually occurring. A series of transcriptional units have been reported by the MATα2 gene product in yeast [25]. Triiodothyronine (T_3) receptor has been found to bind to ERE in a transcriptionally inactive form and to compete for ER binding [26]. Similar competition has been found to occur on T_3-responsive gene between T_3 receptors and v-erb A oncogene product [27]. If GR–Dex complexes can bind to glucocorticoid-responsive element (GRE) in a transcriptionally less active form, some mutation should be present in the GR molecule in SC-3 cells. Cotransfection with the human GR expression vector abolished the inhibitory potency of Dex, suggesting that GR endogenously present in SC-3 cells is mutated. Recently, sequence data on GR in SC-115 have been published [28]. According to this reported result, two kinds of mutations were present in GR molecules: one amino acid substitution from valine to glycine in the first zinc finger portion; and one amino acid (arginine) addition between two zinc fingers. Our recent data revealed only one type of mutation in SC-3 cells (valine to glycine mutation in the first zinc finger portion) (to be published). These findings suggest the possible existence of different GRs among sublines even derived from SC-115 tumor. These heterogenous molecular forms of GR would explain the apparent discrepancy between the present result and the reported observation that Dex can activate the endogenous MMTV gene present in SC-115 cells at 10^{-7} M [29]. Additionally, the molecular mechanism should be examined to explain why an abnormally high concentration of Dex is required to demonstrate the positively regulatory ability of transactivation (Fig. 2).

Modulation of androgen effects by glucocorticoid has been reported in the ductus deferences derived smooth muscle cell line (DDT_1-MF-2) [30]. Growth of DDT_1-MF-2 cells has been shown to be enhanced by androgen, whereas glucocorticoid inhibits their growth. Recent *in situ* hybridization data have demonstrated that glucocorticoid does not inhibit, but facilitate, androgen-dependent accumulation of mRNA coding for heparin-binding growth factor I [31]. Compared with these interesting, but complicated, results, SC-3 cells provide us with more straightforward data on cell proliferation as well as gene expression. Thus, SC-3 cell seem to be a model system suitable for investigation of steroid hormone actions in transformed target cells.

Acknowledgements—We thank Dr R. M. Evans for providing the human GR expression vector. This investigation was supported by grants-in-aid from the Ministry of Education, Tokyo, Japan, from Cancer Research Promotion and from the Hirai Cancer Research Fund.

REFERENCES

1. Jensen E. V., Block G. E., Smith S., Kyser K. and DeSombre E. R.: Estrogen receptors and breast cancer response to adrenalectomy. *Natn. Cancer Inst. Monogr.* **34** (1971) 55–70.
2. Bourgeois S., Gasson J. C.: Genetic and epigenetic bases of glucocorticoid resistance in lymphoid cell lines. In *Biochemical Actions of Hormones* (Edited by G. Litwack). Academic Press, New York, Vol. XII (1985) pp. 311–351.
3. Harmon J. M. and Thompson E. B.: Glucocorticoid resistance in leukemic cells. In *Resistance to Antineoplastic Drugs* (Edited by D. Kessel). CRC Press, Boca Raton, Fla (1988) pp. 385–402.
4. Stevens J., Stevens Y-W. and Haubenstock H.: Molecular basis of glucocorticoid resistance in experimental and human leukemia. In *Biochemical Actions of Hormones* (Edited by G. Litwack). Academic Press, New York, Vol. X (1983) pp. 383–446.
5. Lucas K. L., Barbour K. W., Housley P. R. and Thompson E. A.: Biochemical and molecular characterization of the glucocorticoid receptor of lymphosarcoma P 1798 variants. *Molec. Endocr.* **2** (1988) 291–299.
6. Sibley C. H. and Tomkins G. M.: Mechanism of steroid resistance. *Cell* **2** (1974) 221–227.
7. Bourgeois S., Newby R. F. and Heut M.: Glucocorticoid resistance in murine lymphoma and thymoma cell lines. *Cancer Res.* **38** (1978) 4279–4284.
8. McGuire W. L.: *Progress in Cancer Research and Therapy. Hormones, Receptors and Breast Cancer.* Raven Press, New York, Vol. 10 (1978).
9. Adler S., Waterman M. L., He X. and Rosenfeld M. G.: Steroid receptor-mediated inhibition of rat prolactin gene expression does not require the receptor DNA-binding domain. *Cell* **52** (1988) 685–695.
10. Meyer M-E., Gronemeyer H., Turcotte B., Bocquel M-T., Tasset D. and Chambon P.: Steroid hormone receptors compete for factors that mediate their enhancer function. *Cell* **57** (1989) 432–442.
11. Vaccaro M., Pawlak A. and Jost J-P.: Positive and negative regulatory elements of chicken vitellogenin II gene characterized by *in vitro* transcription competition assays in a homologous system. *Proc. Natn. Acad. Sci. U.S.A.* **87** (1990) 3047–3051.
12. Darbre P. D. and King R. J. B.: Progression to steroid insensitivity can occur irrespective of the presence of functional steroid receptors. *Cell* **5** (1987) 521–528.
13. Minesita T. and Yamaguchi K.: An androgen-dependent mouse mammary tumor. *Cancer Res.* **25** (1965) 1168–1175.
14. Omukai Y., Nakamura N., Hiraoka D., Nishizawa Y., Uchida N., Noguchi S., Sato B. and Matsumoto K.: Growth-stimulating effect of pharmacological doses of glucocorticoid on androgen-responsive Shionogi carcinoma 115 *in vivo* in mice and in cell culture. *Cancer Res.* **47** (1987) 4329–4334.
15. Noguchi S., Nishizawa Y., Uchida N., Yamaguchi K., Sato B., Kitamura Y. and Matsumoto K.: Stimulative effect of physiological doses of androgen or pharmacological doses of estrogen on growth of Shionogi carcinoma 115 in mice. *Cancer Res.* **45** (1985) 5746–5750.
16. Noguchi S., Nishizawa Y., Nakamura N., Uchida N., Yamaguchi K., Sato B., Kitamura Y. and Matsumoto K.: Growth-stimulating effect of pharmacological doses of estrogen on androgen-dependent Shionogi carcinoma 115 *in vivo* but not in cell culture. *Cancer Res.* **47** (1987) 263–268.
17. Lu J., Nishizawa Y., Tanaka A., Nonomura N., Yamanishi H., Uchida N., Sato B. and Matsumoto K.: Inhibitory effect of antibody against basic fibroblast growth factor on androgen- or glucocorticoid-induced growth of Shionogi carcinoma 115 cells in serum-free culture. *Cancer Res.* **49** (1989) 4963–4967.
18. Nakamura N., Nishizawa Y., Matsumoto K., Noguchi S., Terada N., Uchida N. and Sato B.: Both androgen and glucocorticoid induce identical secretory proteins in serum-free culture of Shionogi carcinoma 115 cells. *Jap. J. Cancer Res. (Gann)* **78** (1987) 937–945.
19. Hiraoka D., Nakamura N., Nishizawa Y., Uchida N., Noguchi S., Matsumoto K. and Sato B.: Inhibitory and stimulatory effects of glucocorticoid on androgen-induced growth of murine Shionogi carcinoma 115 *in vivo* and in cell culture. *Cancer Res.* **47** (1987) 6560–6564.
20. Hollenberg S. M., Weinberger C., Ong E. S., Cerelli G., Oro A., Lebo R., Thompson E. B., Rosenfeld M. G. and Evans R. M.: Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* **318** (1985) 635–641.
21. Cohen J., Eccleshall T., Needleman R., Federoff H., Buchferer B. and Marmur J.: Functional expression in yeast of the Escherichia coli plasmid gene coding for chloramphenicol acetyl transferase. *Proc. Natn. Acad. Sci. U.S.A.* **77** (1980) 1078–1082.
22. Herbomel P., Bourachot B. and Yaniv M.: Two distinct enhancers with different cell specificities coexist in the regulatory region of polyoma. *Cell* **39** (1984) 653–662.
23. Cato A. C. B., Miksicek R., Schutz G., Arnemann J. and Beato M.: The hormone regulatory element of mouse mammary tumor virus mediates progesterone induction. *EMBO Jl* **5** (1986) 2237–2240.
24. Ham J., Thomson A., Needham M., Webb P. and Parker M.: Characterization of response elements for androgens, glucocorticoids and progestins in mouse mammary tumor virus. *Nucleic Acids Res.* **16** (1988) 5263–5276.
25. Nasmyth K. and Shore D.: Transcriptional regulation in the yeast life cycle. *Science* **237** (1987) 1162–1170.
26. Glass C. K., Holloway J. M., Devary O. V. and Rosenfeld M. G.: The thyroid hormone receptor binds with opposite transcriptional effects to a common sequence motif in thyroid hormone and estrogen response elements. *Cell* **54** (1988) 313–323.
27. Damm K., Thompson C. C. and Evans R. M.: Protein encoded by v-erb A functions as a thyroid-hormone receptor antagonist. *Nature* **339** (1989) 593–597.
28. Nohno T., Kasai Y. and Saito T.: Novel cDNA sequence possibly generated by alternative splicing of a mouse glucocorticoid receptor transcript from Shionogi carcinoma 115. *Nucleic Acids Res.* **17** (1989) 445.
29. Darbre P. D. and King R. J. B.: Interaction of different steroid hormones during progression of tumor cells to steroid autonomy. *Int. J. Cancer* **40** (1987) 802–806.
30. Syms A. J., Nag A., Norris J. S. and Smith R. G.: Glucocorticoid effects on growth and androgen receptor concentrations in DDT_1-MF-2 cell lines. *J. Steroid Biochem* **28** (1987) 109–116.
31. Harris S. E., Smith R. G., Zhou H., Mannson P-E. and Malark M.: Androgens and glucocorticoids modulate heparin-binding growth factor I mRNA accumulation in DDT1 cells as analyzed by *in situ* hybridization. *Molec. Endocr.* **3** (1989) 1839–1844.