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Two naturally-occurring isoforms and their expression of a glucocorticoid receptor gene from an androgen-dependent mouse tumor

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We have isolated cDNAs encoding the glucocorticoid receptor from an androgen-dependent mouse tumor, Shionogi Carcinoma 115. The nucleotide sequence of the receptor revealed two different forms, designated as SC-GR I and SC-GR II. Both forms have a one-base substitution in the DNA binding domain of the wild-type mouse glucocorticoid receptor. Furthermore, SC-GR II has a three-base insertion in the interfinger region of this domain. By expressing the receptor cDNAs in cultured cells with MMTV-CAT reporter plasmid, SC-GR II was found to have about half of the activity of the wild-type mouse glucocorticoid receptor induced by either physiological or pharmacological doses of dexamethasone.

Glucocorticoid receptor; cDNA cloning; CAT assay; Shionogi Carcinoma 115; Androgen-dependent mouse tumor

1. INTRODUCTION

Recently, almost all of the steroid and thyroid hormone receptor cDNAs have been cloned [1-4]. Because they have highly-conserved amino acid sequences in the middle part of the coding sequence, the receptors constitute the co-called 'steroid and thyroid hormone receptor superfamily' [1,2]. The DNA binding domain, the most highly conserved region, is considered to form two zinc-coordinated fingers based on the structural motif first described for the *Xenopus* gene transcription factor, TF III A [5,6], and is presumed to have the ability to recognize its own hormone responsive elements [7].

Shionogi Carcinoma (SC115) is a mouse mammary tumor dependent on androgen for its growth [8]. However, its growth is contradictorily regulated by glucocorticoid [9-12]. In order to define the role of the glucocorticoid receptor (GR) in the growth response to glucocorticoid, we cloned SC115 GR cDNA and determined its nucleotide sequence.

The present work shows that the SC115 GR sequence has two different forms [13]; one with a one-base substitution (SC-GR I) and the other with an additional three-base insertion (SC-GR II) in the DNA binding domain of the wild-type mouse GR reported previously [14]. When expressed in COS-1 cells, SC-GR II showed about half of the wild-type mouse GR in a CAT assay for GR, responding to either physiological or pharmacological doses of dexamethasone.

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2. MATERIALS AND METHODS

2.1. *Preparation of a SC115 cDNA library*

SC115 was maintained in mature male DS mice by serial subcutaneous transplantation for three to four week intervals [15].

Polyadenylated RNA was selected from the total RNA of the SC115 tumor by oligo(dT)-cellulose chromatography (Pharmacia). Double-stranded cDNA was synthesized with reverse transcriptase and DNA polymerase I using oligo(dT)17 as a primer [16]. The cDNA was inserted into λgt11 and packaged in vitro [17]. The cDNA library contained about 10^6 independent recombinants.

2.2. *Isolation of glucocorticoid receptor cDNA*

Screening of the cDNA library was accomplished by plaque hybridization using a 143-bp *Hin*dIII-*Sph*I fragment of rat glucocorticoid receptor cDNA as a probe [18]. The hybridization was carried out in a solution containing 50% formamide, 5× Denhardt, 5× SSPE, 0.1% SDS, 100 μl/ml denatured salmon sperm DNA and ^{32}P-labeled 143-bp oligonucleotide probe at 42°C for 16 h. After being washed in 2× SSPE and 0.1% SDS three times for 15 min at 42°C to remove excess probes, the filters were autoradiographed (Kodak XAR film) for 24 h at −70°C with an intensifying screen [17].

Positive clones were isolated, mapped with restriction enzymes, and then subcloned into Phagescript SK (Stratagene). The DNA sequence was determined by the dideoxy chain-termination method using the M13 universal primer [19].

2.3. *Construction of pSV2S-GR II and its expression*

pSV2S-GR II was constructed by replacing *Apa*I and *Pst*I fragments in pSV2Wrec [14], the full functional plasmid of wild-type mGR, with the cDNA of SC-GR II. 5 μg of pSV2Wrec or pSV2S-GR II and 20 μg of pMSG-CAT (Pharmacia), a reporter plasmid containing the chloramphenicol acetyl-transferase (CAT) gene and the dexamethasone-inducible MMTV promoter, were co-transfected into COS-1 cells (provided by the Japanese Cancer Research Resources Bank) by the calcium phosphate co-precipitation technique (Mammalian Transfection Kit, Stratagene) [20]. The DNA-calcium-phosphate mixtures were co-transfected into 5×10^5 cells/100 mm dish COS-1 cells prepared more than 24 h earlier and incubated overnight in 10 ml Dulbecco modified Eagle medium (DMEM) with 10% bovine serum albumin at 35°C under a 4% CO_2 condition. The cells

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were split into several dishes containing 2×10^3 cells, and 10^{-4} M or 10^{-6} M dexamethasone (Sigma) in ethanol was dropped into the plate after an additional 24 h-incubation in 5 ml of DMEM with 10% charcoal-treated serum (final 10^{-6} M or 10^{-8} M). The same volume of ethanol without dexamethasone was added to the control. The cells were harvested after 24 h-incubation and used for measuring CAT activity. The CAT assay was performed following the Novel Diffusion-Based CAT assay protocol of Dupont-New England Nuclear Research Products [21]. Samples were counted in an ALOKA 3100 liquid scintillation counter for 2 min at room temperature after 0, 2 and 4 h incubation.

3. RESULTS

3.1. *Cloning and sequencing of SC115 GR cDNA*

By screening 10^6 plaques of the SC115 cDNA library, we could obtain 33 positive clones. We identified 17 overlapping glucocorticoid receptor cDNA clones by restriction mapping (Fig. 1A).

The sequence analysis revealed a 2349 bp open reading frame of SC115 GR cDNA. The nucleotide sequence of SC115 GR cDNA was exactly the same as that reported for mouse lymphoma cells [14], except for a one-base substitution at 1310 and a three-base insertion between 1372 and 1373 (Fig. 1B). Both changes are in the coding region of the Cys-rich DNA binding domain of the glucocorticoid receptor.

The one-base substitution at 1310 from T to G accompanying amino acid substitution from Val to Gly was observed in all clones. The three-base insertion, GTA, between 1372 and 1373 was observed in two clones out of 17 independent clones. The insertion of the three-base resulted in the addition of Arg between Gly-458 and Glu-459 without a shift in the transcription frame (Fig. 1B). We designated the GR with the one-base substitution as 'SC-GR I', and the GR with the three-base addition as 'SC-GR II'.

Table I

Comparison of dexamethasone-induced CAT activity between pSV2Wrec and pSV2 S-GR II

Receptor Plasmid	CAT activity (fmol/cell · min)			
	10^{-6} M dexamethasone		10^{-8} M dexamethasone	
pSV2Wrec	2.69 ± 0.65	54%*	2.56 ± 0.80	48%*
pSV2S-GR II	1.46 ± 0.13		1.24 ± 0.15	

Mean ± SE of 5 independent determinations. *Significantly different from pSV2Wrec at $P < 0.05$ % = percentages of the mean of pSV2Wrec.

3.2. *Expression of SC115 GR cDNA*

A diffusion-based CAT assay demonstrated that SC115 GR cDNA encoded a functional glucocorticoid

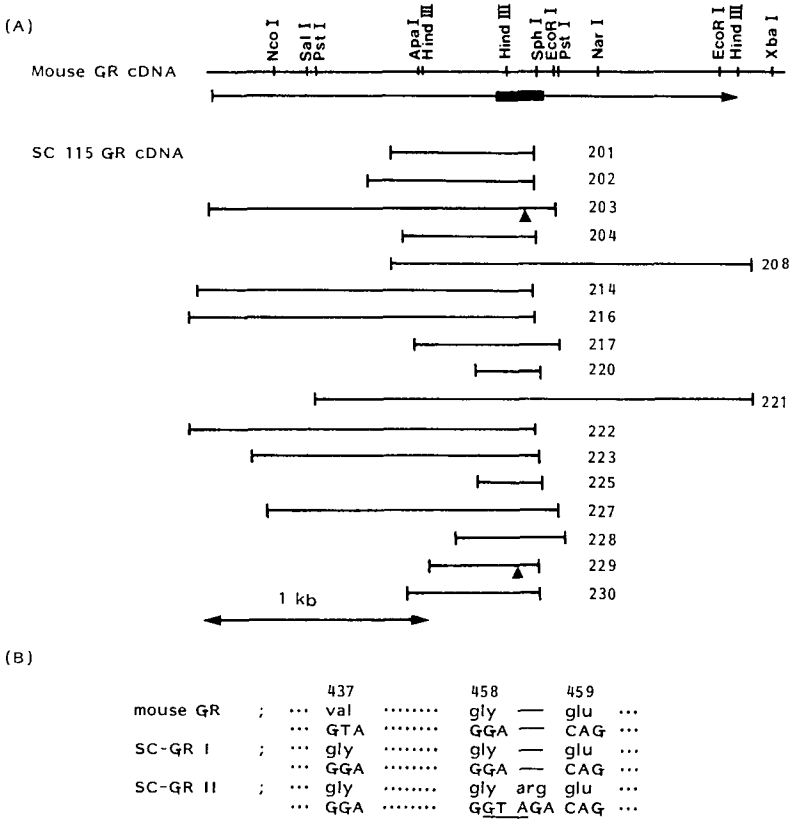

Fig. 1. Cloning of SC115 GR cDNA comparison with mouse GR cDNA. (A) Seventeen overlapping clones of SC115 GR cDNA; (■) shows DNA binding domain of mGR; (▲) shows the position of the 3-base insertion. (B) Comparison of the nucleotide and amino acid sequence of SC115 GR with mGR (DNA binding domain). Only those residues that differ from mouse GR are shown and numbered from the first base of the initiation codon. Underlined is the nucleotide sequence of the 3-base insertion.

receptor. As a control, pSV2CAT [22] was used for monitoring the transfection efficiency. The results of the CAT assay are shown as the means obtained in 5 independent transfections (Table I). According to a *t*-test, the CAT activity of pSV2S-GR II was significantly lower than that of pSV2Wrec. The CAT activity of pSV2S-GR II was about half of that of pSV2Wrec when induced by either 10^{-6} M (pharmacological doses) or 10^{-8} M (physiological doses) of dexamethasone.

4. DISCUSSION

We demonstrated the presence of two forms of glucocorticoid receptor in the mouse tumor, SC115 GR. The amino acid at 437 in GR so far examined is commonly Gly [14,18,23]. Only in the mouse GR, is Val exceptionally substituted for Gly-437. SC-GR I has alsmost the same activity as wild-type mGR [14]. As for SC-GR II, according to the genomic organization of the human estrogen [24] and chicken progesterone receptor gene [25], the inserted region is matched to the splicing junction between the second and third exons. Since these splicing junctions should be also conserved in GR, three bases of donor sites on the primary transcript may be retained after splicing.

Proliferation of SC115 is known to be stimulated by androgen [8]. Moreover, it has been reported that the growth of SC115 cells is also stimulated by pharmacological, but not physiological doses of glucocorticoid both in vivo and in cell culture. Dexamethasone at 10^{-8} M inhibits the proliferation of these cells by 30% but stimulates them by 235% at 10^{-6} M in the temporary absence of testosterone [9]. This interesting proliferation property was investigated in detail in a cell line derived from SC115 in a serum-free medium by Hiraoka et al. [11]. They showed that the stimulation induced by a high dose of testosterone was inhibited by the addition of dexamethasone but it was enhanced by dexamethasone if induced by a low dose of testosterone. The dual effect of dexamethasone on this tumor may be related to SC-GR and their regulation processes. However, our CAT assay results demonstrated that pSV2S-GR II, the expression plasmid of SC-GR II, had about half of the activity of pSV2Wrec, wild-type mouse GR, in both higher (10^{-6} M) and lower (10^{-8} M) doses of dexamethasone. Our CAT assay data could not explain the contradictory effects of dexamethasone on the growth of SC115.

Research during the past few years has revealed that certain members of the steroid and thyroid hormone receptor superfamily and its mutant forms may be related to malignant transformation and certain malignant states of various tumors [26]. As for the mouse glucocorticoid receptors, certain mouse lymphoma cells resistant to dexamethasone are known to contain mutant glucocorticoid receptors due to a single amino acid substitution [14]. Another report has shown that the human estrogen receptor cDNA clone from MCF-7 cells has a Gly to Val mutation in the hormone binding domain and a decreased affinity for estradiol [24]. On the other hand, alternative splicing of thyroid hormone receptor genes is a ubiquitous mechanism for generating products with different functions derived from a single gene [27]. The existence of two forms of GR, even if in a minor population of SC115 cells and by miss-splicing, may suggest the different roles of the glucocorticoid receptors. Recent studies on human GR-mediated repression using a cAMP-inducible chorionic gonadotropin α-subunit promoter, which is negatively regulated by glucocorticoid, revealed that the repression manner requires DNA and ligand binding domains [28,29]. In particular, substitution of a single amino acid of the DNA binding domain showed that 18 out of 19 mutants have parallel effects on activation and repression, but one mutant, a substitution of the linker region between the two Zn-fingers, had a repressor function but no activator function. In SC-GR II, the linker region of the DNA binding domain has an additional basic amino acid (Arg) which may change the ability to distinguish GRE from other hormone responsive elements [30,31]. Further study is required to determine whether SC-GR II has the ability to recognize other GRE related to negative transcriptional regulation by glucocorticoid actions.

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