ISOLATION AND CHARACTERIZATION OF HUMAN TR3 RECEPTOR: A MEMBER OF STEROID RECEPTOR SUPERFAMILY

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Summary—Complementary DNAs (cDNAs) encoding a member of steroid receptor super-family, named TR3 receptor, were isolated from a human prostate λgt11 cDNA library on the basis of homology of oligonucleotide probes to the DNA-binding domain common to members of the steroid receptor super-family. Expression of TR3 receptor cDNA produced a 64 kDa DNA-binding protein in a rabbit reticulocyte lysate. Nucleotide sequence analysis showed that TR3 receptor cDNA contains two regions of sequences which correspond to the DNA- and hormone-binding domains of members of the steroid receptor super-family. The amino acid sequences in the hormone-binding domain of the TR3 receptor shares about 20% homology with estrogen receptor and less than 15% homology with other known steroid receptors. The DNA-binding domain of the TR3 receptor has about 55% homology with all other known steroid receptors. TR3 receptor had 86% nucleotide and 91% amino acid sequence homology with mouse NUR/77, suggesting that TR3 receptor may be a human homologue of mouse NUR/77 gene product.

INTRODUCTION

The steroid receptor super-family includes receptors for steroid hormones, thyroid hormones, retinoic acid and other receptor-like proteins for as yet unidentified ligands [1–3]. Members of this super-family have in common structural features which participate in DNA-binding and ligand-binding in the regulation of specific gene expression [4–7]. Based on the hybridization of oligonucleotide probes homologous to a highly conserved sequence in the DNA-binding domain, we have previously isolated cDNAs encoding three members of this family: androgen receptor [8–10], TR2 receptors [2] and TR3 receptor [11]. We report here the strategy to isolate the full-length of TR3 receptor cDNAs, the nucleotide and deduced amino acid sequences of TR3 receptor, and sequence comparison between TR3 receptor and other known steroid receptors. These results suggest that the TR3 receptor may be structurally related to the mouse NUR/77 gene product, a protein which may be involved in the regulation of cell division and growth [12].

Materials

[a-35S]dATP (1000 Ci/mmol) and L-[35S]methionine were from Amersham. DNA sequencing kit with sequenase was obtained from United States Biochemical. Human prostate cDNA library was constructed as described elsewhere [9, 10]. All restriction enzymes were the products of Bethesda Research Laboratories. NUR/77 probe was provided by L. Lau of Northwestern University.

Isolation of human TR3 receptor cDNA clones

In the process of screening a human testis and prostate λgt11 cDNA libraries for androgen receptor cDNA [8–10], two new classes of steroid receptor-like cDNAs were isolated: TR2 receptors [2] and TR3 receptor [11]. In general, clones were selected and differentiated using oligonucleotide probes specific for various steroid receptors. The cDNA libraries were initially screened with 5'-end 32P-labeled 41-base oligonucleotide probes designed for homology with highly conserved nucleotide sequences in the DNA-binding domain of known steroid receptors. Positive clones were then screened with 5'-end 32P-labeled 24-base oligonucleotides specific for the known steroid receptors. One positive clone (TR3 receptor) which was found to hybridize strongly to mouse BALB/c 3T3 cell NUR/77 probe [12] was used as a
probe to further screen a human prostate cDNA library for the full length of TR3 receptor cDNAs. DNA inserts in the positive TR3 receptor clones were analyzed by restriction mapping and subcloned into M13 vectors for dideoxy sequence analysis [13] and into pGEM-3Z vector for in vitro expression.

**In vitro transcription/translation of TR3 receptor**

pGEM-3Z vector (20 μg) containing 2.5 kb TR3 DNA segment was linearized with restriction enzyme Sca I. Transcription and translation were carried out as described before [9, 10]. The molecular weight of the [35S] methionine-labelled translation products was determined by SDS-PAGE. For the steroid binding assay, 5 nM tritiated steroid hormone was incubated with lysate in the final volume of 100 μl. [3H]-Steroid binding was measured by the hydroxypapatite-filer method [14, 15].

**RESULTS AND DISCUSSION**

The strategy which allowed us to clone full length human and rat androgen receptor cDNAs [8–10] led to the isolation of two new classes of steroid receptors: TR2 receptors and TR3 receptor [2, 11]. The deduced amino acid sequence from cDNA showed that the DNA-binding domain of TR3 receptor has a high degree of homology with the DNA-binding domains of other known members of the steroid receptor super-family. The positions of 26 amino acids (9 Cys, 3 Lys, 3 Arg, 2 Tyr, 2 Gly, 2 Phe, 1 Met, 1 Asp, 1 His, 1 Ala, 1 Ser) in the DNA-binding domain are identical among all known members of the steroid receptor super-family (Fig. 1). The DNA-binding domain of the TR3 receptor has about 55% homology with all other known steroid receptors. These findings suggest that the TR3 receptor, like steroid receptors, may be a transcriptional regulator which controls gene expression.

Nucleotide sequence analysis showed that cDNA for TR3 receptor is 2473 base pairs in length (Fig. 2). The open reading frame between the first ATG and terminator TGA encodes 598 amino acids with a calculated molecular weight of 64 kDa. This calculated molecular weight matches well with in vitro translated TR3 receptor protein (Fig. 3). The second detectable protein product with molecular weight of 60 kDa may represent the open reading frame between second ATG (amino acid number: 35) and terminator TGA. In the 3'-nontranslated region of TR3 receptor cDNA, a eukaryotic polyadenylation signal AATAAA is located 17 bases upstream of the poly(A) tail. The 5' region of TR3 receptor, like androgen receptor or progesterone receptor, is characterized by a high proline content which may make the region flexible to conformational changes [10, 16]. In the putative hormone-binding domain, which consists of about 200 amino acids near the carboxyl terminal of steroid receptors [1–3], the homology between TR3 receptor and estrogen receptor was about 20%, whereas the homology between TR3 receptor and androgen receptor, progesterone receptor, glucocorticoid receptor, mineralocorticoid receptor, and TR2 receptor was less than 15% (Fig. 4).

To study the steroid-binding activity of the protein encoded by the TR3 receptor cDNA, in vitro synthesized protein was incubated with all major classes of steroids, including androgen, progesterone, glucocorticoid and estrogen. We have failed to show any significant binding with these steroids. Because the

Fig. 1. Amino acid sequence alignment of the cysteine rich DNA-binding domain for the TR3 receptor and other human steroid receptors: androgen receptor (AR), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), progesterone receptor (PR), estrogen receptor (ER), TR2 receptor. The numbers represent the positions of amino acid residues in the individual receptors. Common residues are boxed.
Fig. 2. cDNA sequences and deduced amino acid sequences of human TR3 receptor. The nucleotide sequences and the deduced amino acid sequences are numbered on the left. The nucleotide and amino acid sequences in the DNA-binding domain are boxed and eukaryotic polyadenylation signal AATAAA is underlined.

homology between TR3 receptor and other steroid receptors in the putative hormone binding region is low (Fig. 4), the function of TR3 translated protein may be dependent on binding to an unidentified, non-steroidal ligand.

Hybridization experiments showed that NUR/77, a cDNA derived from mRNA of serum-stimulated mouse BALB/c 3T3 cells [12], hybridized to TR3 receptor cDNA under conditions of high stringency. Nucleotide sequence analysis revealed that TR3 receptor displays 86% nucleotide and 91% amino acid sequence homology with NUR/77, disregarding three extra residues in NUR/77, suggesting that TR3 receptor may represent the human homologue of NUR/77 [17]. Transcription of NUR/77 is rapidly activated by the addition of fetal calf serum, PDGF
Fig. 3. SDS-PAGE analysis of in vitro transcription/translation of TR3 receptor. The standard molecular weight proteins used were carbonic anhydrase (30 kDa), ovalbumin (46 kDa), bovine serum albumin (69 kDa), and phosphorylase b (92 kDa).

Fig. 4. Amino acid comparison between human TR3 receptor, TR2 receptor, ER, GR, AR, PR and MR in their DNA-binding domain and hormone-binding domain.

or FGF to quiescent cells [12, 17]. The homology of TR3 receptor with the NUR/77 gene product, which may have an important role in the regulation of cell division and growth, has significant implications for the function of TR3 receptor in the normal and neoplastic prostate.

Acknowledgements—We thank L. Lau of Northwestern University for the NUR/77 plasmids. We also thank C. Whelan and L. Acakpo-Satchivi for their expert technical assistance. This work was supported by Coleman and University of Chicago Cancer Research Foundation, Grant IN-41-30 from American Cancer Society and Grant DK37694 from National Institute of Health.

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