MOLECULAR CLONING OF THE CHICKEN GROWTH HORMONE RECEPTOR GENE AND DEMONSTRATION OF THE GENE MUTATION IN SEX-LINKED DWARF CHICKENS

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SUMMARY

Chicken growth hormone (GH) receptor gene was isolated from a genomic library, and its promoter region and several exons have been sequenced. The coding fragments span about only 10 kilobase, unlike the human GH receptor gene, which spans at least 87 kilobase. Sex-linked dwarfism in chicken is a recessive genetic disorder that is characterized by undetectable levels of hepatic GH-binding activity and a mutation in the GH receptor gene. With the region specific DNA probes from our genomic cloning, characterization of the GH receptor gene indicates that sex-linked dwarf chickens have a deletion of approximately 2 kilobase portion in the intracellular domain of the receptor gene. These direct evidence demonstrate that sex-linked dwarfism could result from a defeat in the structural gene of the GH receptor gene in chicken.

INTRODUCTION

In general, animal growth-promoting effects of GH is mediated via the insulin-like growth factors(IGF). IGF is mainly produced in the liver cells, but the liver cells dramatically produce and secret IGF only in response to the signal of circulating GH, which is transduced by GH receptor (Daughaday and Rotwein, 1989). GH receptor is known to be a member of the cytokine receptor family, which includes the prolactin, erthropoietin, interleukin2,4,6 and granulocyte macrophage colony stimulating factor receptors. These have common disulphide motifs in the extracellular region for hormone binding. Chicken GH receptor is quite similar in size and structure to the mammalian GH receptor, which contains a typical signal sequence of 16 amino acids, and the mature peptide consists of 592 amino acids. A single 24 amino acids membrane spanning domain locates at amino acids 238- 261. The homology of amino acid sequences between chicken GH receptor and mammalian GH receptor is in a range of 50- 58%°. (Leung et al, 1987; Burnside et al, 1991).

Sex-linked dwarfism (SLD) is a recessive mode of inheritance that is characterized by rather high circulating levels of GH but low levels of IGF and a failure to detect hepatic GH-binding activity (Leung et al., 1987). Recently a restriction fragment length polymorphism (RFLP) and an aberrently sized transcript of chicken GH receptor gene have been identified in one population of SLD chickens (Burnside et al., 1991; 1992). These studies suggest that mutation of the GH receptor gene accounts for the SLD phenotype. In order to define the molecular biological function of the chicken GH receptor and to map the mutation region of GH receptor gene, we have isolated chicken GH receptor gene from a genomic library. With the region specific DNA probe from our genomic cloning, characterization of the GH receptor gene indicates that SLD in Rongda Brown chickens have a deletion of approximately 2 kb portion in the intracellular domain of the gene.

MATERIALS AND METHODS

Liver or blood samples from 35-week-old male and female chickens (obtained from an egg-type normal line and an egg-type SLD line of Rongda Brown, Commercial Poultry Breed Farm of Beijing Agricultural University, Beijing) were collected and stored in a frozen container.

Chicken genomic library was constructed by using λEMBL 3 vector followed the standard method (Maniatis et al., 1982). The library was screened with ^{32}P-labbeled chicken GH receptor cDNA, and the hybridization was performed at 42 °C in 50% formamide/0.75 M NaCl/ 0.075M trisodium citrate, and then the filters were washed at 42 ° C in 0.03M NaCl/ 0.003M trisodium citrate/ 0.1% SDS. DNA sequencing was performed by the dideoxy chain-termination method using genomic fragments subcloned into pGEM vectors (Promega).

Genomic DNA was prepared from normal or SLD blood. 8 μg DNA was digested with restriction enzymes, electrophoresed on a 0.8% agarose gel, transferred to nylon membrane, hybridized and washed as above described procedures. Probes for genomic hybridization were labelled with ^{32} P by a random prime extension method. To generate the region specific probes, according to the restriction map the full genomic cloning of chicken GH receptor was cut into several DNA fragments which correspond to promoter domain, extracellular and intracellular domain respectively.

RESULTS

Of the 2 x 10^5 recombinant plaques screened, three positive clones , named λcGHR 1, λcGHR 2,λcGHR 3, were obtained from the genomic library. Restriction endnuclease analysis of DNA isolated from these clones showed that they are all harbouring a 2.5 kilobase (kb) EcoR I fragments and overlapping each other. The complete restriction map of these clones was constructed with Sal I, EcoR I, BamH I and Hind III enzyme (Fig. 1). The clone λcGHR 1 was chosen for further mapping. 25 mer oligonucleotide reduced from the 5' untranslated domain of chicken GH receptor cDNA (Burnside et a., 1991) was chemically synthesized as probe for determining the promoter region of chicken GH receptor gene. 800 base pairs (bp) DNA fragments from the intracellular domain and 3' untranslated domain of chicken GH receptor cDNA was generated as probe for localizing the termination region of chicken GH receptor gene. The results of mapping indicated that 2.5 kb EcoR I fragment contains the promoter region of the gene and the gene sequence is most like to end in the 0.8 kb EcoR I fragment.

1.3 kb EcoR I fragment, 2.5 kb EcoR I fragment, 5.8 kb EcoR I fragment and 0.8 kb EcoR I fragment from the insertion of λcGHR 1 clone were subcloned into pGEM vector respectively and subjected to performing oriently deletion for sequencing. The nucleotide sequence of 2.5 kb EcoR I fragment has been completely determined (Fig.2), and the possible Goldberg-Hogness boxes have been identified. The partial nucleotide sequence of 0.8 kb EcoR I fragment showed consensus sequence AATAAA for poly A tailing signal. However, since the full sequence of chicken GH receptor gene is still on the way, the nature organization of the gene has not been figured out so far.

The cDNA of chicken GH receptor was used to probe genomic blots of DNA from control and SLD chickens of Rongda Brown. The restriction pattern of EcoR I-digested DNA exhibits a polymorphism, where the predominant hybridizing band is approximately 5.8kb and 2.5 kb in control birds but 4.0 kb and 2.5 kb in SLD chickens (Fig.3). With 25 mer olignucleotides synthesized and 2.5 kb EcoR I fragment isolated from λcGHR 1 clone as probe to repeat this blot experiment, only 2.5 kb hybridizing band can be found and the polymorphism seems to disappear. The Hind III digest shows approximately 2.4

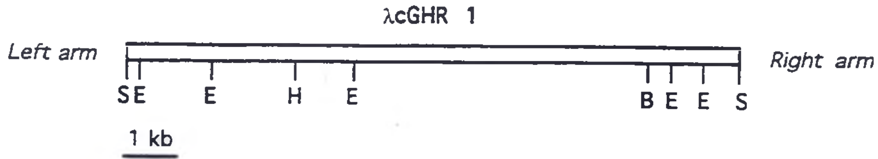

Fig. 1. Restriction map of chicken GH receptor genomic clone λcGHR 1. Restriction enzymes used in subcloning are: S (Sal I), E (EcoR I), H (Hind III), B (BamH I).

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taaataccatattataaaatgctctgcgaattc

Fig. 2. DNA sequence of 2.5 kb EcoR I genomic clone which probably encodes the promoter region of chicken GH receptor gene.

kb hybridizing band in normal chickens and about 3.1 kb in SLD birds (Fig.3). No significant difference was detected in the band pattern of Hinf I digest (7.0 kb and 3.0 kb) and Taq I digest (3.2 kb) with control and SLD chickens.

DISCUSSION

We have cloned the chicken GH receptor gene and determined the partial nucleotide sequence of this gene. With the information of GH receptor genomic clones, The receptor gene of SLD chickens have been examined, and the results demonstrated that a 2kb DNA deletion in receptor allele that probably removes the coding region of intracellular domain of receptor, which would damage the normal function of receptor. Indeed, the process of receptor for GH signal transduction dramatically depends on the intracellular domain to promote the phosphorylation of receptor itself on tyrosyl residues or generate a site interaction with intracellular substrates or effector proteins (Li and Chen, 1994). Thus, these data provide convincing evidence that SLD can result from defects in the structural gene for the GH receptor, although the complete nucleotide sequence of the normal receptor gene has not been worked out.

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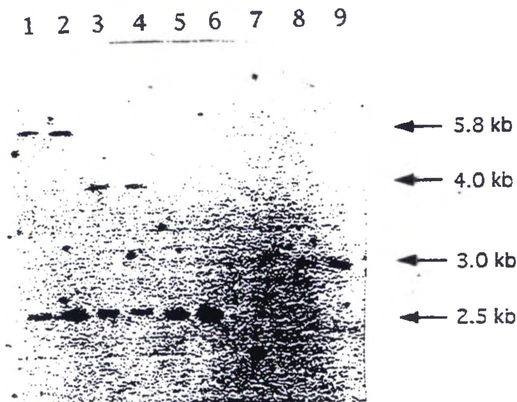

Fig. 3. Autoradiograph of genomic Southern blot analysis. 1-2, Genomic DNA of normal chickens digested with EcoR I; 3-4, Genomic DNA of SLD chickens digested with EcoR I; 5-6, Genomic DNA of normal chickens digested with Hind III; 7-9, Genomic DNA of SLD chickens digested with Hind III.