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研究室で撮影した本人のスナップ写真、及び発表論文等のコピーを添付

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研究テーマ 高血圧症に関するゲノム解析

2．日本滞在日程

平成10年8月25日 成田到着

8月26日～12月15日 高血圧症原因遺伝子発見のためのＢＡＣクローンのスクリーニング、ＢＡＣクローンの一部整列

12月16日～12月19日 日本分子生物学会参加

12月20日～平成11年2月24日 整列ＢＡＣクローンの一部シーケンシング、シーケンスのコンピュータ解析による候補遺伝子の探索

2月25日 帰国

3．研究報告

別紙書式を参考に、報告本文4000字以上で報告して下さい（枚数自由・ワープロ使用）

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研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。

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研究テーマ: 高血圧症に関するゲノム解析

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要旨：インスリン非依存性糖尿病患者の家系分析によれば、高血圧に関する遺伝的素因が８番染色体のＬＰＬ遺伝子の近傍（8p22）に存在することを示唆している。本研究ではこの結果を確認し高血圧関連遺伝子をクローニングするために、ＬＰＬ近傍の１３個のＳＴＳマーカーを用いて、慶應ＢＡＣライブラリーをコロニーハイブリダイゼーションでスクリーニングした。得られた２９個のＢＡＣクローンをサザンブロットおよびＰＣＲを併用して整列した上で、６個の重なり合うＢＡＣクローンを選定しそれらを用いてエキソントラッピングを行った。３０個のエキソンが得られたのでそれぞれシーケンスを決定しデータベースへのホモロジーサーチを行った。その結果、１個はＬＰＬ遺伝子由来のエキソンであったが、他の２９個は未知のシーケンスであった。本研究によって、8p22領域に存在する新規遺伝子のクローニングが可能となり、高血圧関連遺伝子を発見する基盤ができた。

Key Words: エキソントラッピング、ＢＡＣライブラリー、８番染色体、高血圧

Hypertension is a common complex disease characterized by quantitative trait under polygenic control. The identification of genes responsible for high blood pressure is of major importance, because it provides a mechanistic classification and guides therapy for the primary abnormality. Wu *et al* studied the distribution of blood pressure in 48 Taiwanese families with noninsulin-dependent diabetes mellitus and conducted quantitative sib-pair linkage analysis with candidate loci for blood pressure, insulin resistance and lipid metabolism. They obtained significant evidence for linkage of systolic blood pressure to a genetic region at or near the lipoprotein lipase (LPL) gene on chromosome 8p22[1]. As a step toward identifying the pathogenic gene of hypertension, I screened BAC clones from Keio human BAC contig covering markers D8S1715-D8S1949-D8S282. Furthermore, I performed exon trapping and sequencing of the obtained exon-like fragments. This information will aid in the isolation of candidate genes involved in the pathogenesis of hypertension and other disorders mapped to the 8p22 region.

MATERIALS AND METHODS

Human BAC library. The Keio BAC library was constructed by Asakawa *et al.* and reported previously[2].

Synthesis of oligonucleotide probe. The sequence information on 13 STS markers located on chromosome 8p22 was downloaded from the database of the

Whitehead Institute at MIT. They were used as probes for hybridization and PCR amplimers.

Colony hybridization using high-density replica (HDR) filters. The 3072 BAC clones were blotted onto a 12 x 8 cm Biodyne nylon membrane by a Biomek 2000 workstation (Beckman). DNAs were fixed to membranes according to a microwave/proteinase k colony lysis procedure[3]. The end-labeled probe reaction was followed by mixture containing 0.1 pmol each of 13 STS marker oligonucleotides (forward amplimers), 6.5 μCi of [γ-^{32}P] ATP (5000 Ci/mol, AA0018 Amersham), and 1 unit of T4 polynucleotide kinase (Takara) in the supplied buffer and was incubated at 37°C for 1 hr. The unincorporated [γ-^{32}P]ATP was removed with a Sephadex G-50 spun column. Prehybridization was performed with a solution of 5 x SSC, 5 x Denhardt's, 0.5% SDS, 0.5 mg/ml denatured ssDNA at 55°C for overnight. The labeled probes were added to the hybridization solution and incubated for 72 hr at 55°C. The filters were washed three times with 2 x SSC and 0.5% SDS for 15 min at room temperature, followed by two washing for 30 min at 55°C, and finally washed with 0.1 x SSC and 0.1% SDS for a few min at room temperature. The filter was exposured against Fuji imaging plate for 72 hr. The autoradiograms were obtained with a BAS 2000 Bio Imaging Analyzer (Fuji, Japan).

PCR screening. 29 BAC clones were amplified by PCR with 13 STSs respectively.

Southern blot analysis. DNA from 29 BAC clones which cover the 13 markers near LPL locus was digested with *Hin*dIII, separated by 0.7% agarose gel electrophoresis and transferred onto a nylon membrane (NEN). *Hin*dIII-digested DNA was labeled with [α-^{32}P]dCTP by a random priming method and hybridized to the Southern blot filter of BAC DNA.

Exon trapping. Exon trapping was performed using an exon trapping system (GIBCO, BRL) as recommended by the supplier. DNAs of six BAC clones (1220-D6, 1689-F4, 1961-C5, 1460-H10, 1381-A1 and 2048-D6) were digested with *Pst*I and subcloned into *Pst*I-digested pSPL3 vector. Transformed colonies resistant to ampicillin were pooled and plasmid DNA was purified. Recombinant plasmids were transfected into COS-7 cells using FuGENE 6 transfection reagent (BOEHRINGER, MANNHEIM). After 48 hr, total RNA was purified from COS-7 cells, reverse-transcribed and PCR-amplified using vector-specific primers. The PCR products were subcloned into pAMP10 using the uracil DNA glycosylase (UDG) cloning method as recommended by the supplier.

DNA sequencing.. The trapped sequences were amplified directly from each colony by PCR using cassette-specific primers, purified by QIAquick-spin columns (QIAGEN), and then subjected to DNA sequencing. The sequencing

reaction was performed using Taq DNA polymerase and Fluorescence-labeled dideoxy-nucleotides in a BigDye terminator cycle sequencing kit (Perkin-Elmer). The DNA sequence was then determined using an automatic DNA sequencer (ABI, Model 377). The nucleotide homologies were analyzed using BLASTN search of the non-redundant databases.

RESULTS AND DISCUSSION

We attempted to construct a BAC contig map to cover the region corresponding to quantitative trait locus of human blood pressure at chromosome 8p22. From BAC library, 29 BAC clones were selected by colony hybridization with labeled 13 STSs near LPL locus. We then performed PCR with the same STSs to conform the BAC clones containing human DNA inserts. To find neighboring clones, we used Southern hybridization with each BAC clones as the probe. After a series of experiments we were able to construct the BAC contig covering markers D8S1715-D8S1949-D8S282.

To isolate transcribed sequences from this region, we performed exon-trapping using 6 BAC clones, and isolated 30 putative exon fragments. These exons ranged from 57 bp to 261 bp in size. Homology search with nucleotide sequence database revealed that one exon is derived from gene LPL located in this region. 18 exons matched with partial EST sequences without known function. 4 exons matched with exon sequences that were independently trapped by other investigators. 7 exons had no considerable homology with the sequences deposited in database. We found 18 exons to be present in human liver, thymus, brain, placenta, retina and testis cDNA libraries. These exons reported here will be useful resources for isolation of candidate genes involved in the pathogenesis of hypertension as well as other novel genes located in the 8p22 region.

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