Organization, Structure, Chromosomal Assignment, and Expression of the Gene Encoding the Human Endothelin-A Receptor*

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We have isolated and characterized the gene for the human endothelin-A receptor. Southern blot analyses demonstrated a single copy gene for the receptor. The gene spans more than 40 kilobases and contains eight exons and seven introns. Intron 1 exists in the 5'-noncoding region, and introns 2-7 occur in the coding domain. The locations of introns 2-7 exist before or after the regions encoding the membrane-spanning domains. The transcription start site, determined by primer extension experiments, is 502 base pairs upstream of the methionine initiation codon. The 5'-flanking region lacks a typical TATA box but contains a potential SP-1-binding site 27 base pairs upstream of the transcription start site. Using human-rodent somatic hybrid cell DNA, the gene was assigned to human chromosome 4. Northern blot analyses revealed a 4.3-kilobase mRNA in a wide variety of human tissues, at the highest level in the aorta and at a substantial level in the cultured human mesangial cells.

This is the first report of cloning of a gene for a member of the endothelin receptor family. The present study should give a clue to the discovery of possible disorders of the endothelin-A receptor, as well as facilitate the elucidation of the mechanisms by which the gene expression is regulated.

Endothelin-1 (ET-1) was initially identified as a potent vasoconstrictor peptide with 21-amino acid residues produced by cultured porcine aortic endothelial cells (1). Cloning and sequence analyses of the endothelin genes revealed that ETs comprise a peptide family consisting of three isopeptides, ET-1, ET-2, and ET-3, which distribute in a wide variety of tissues (2-7).

Pharmacological studies and binding experiments have suggested the existence of multiple ET receptor (ETR) subtypes (6-8). In 1990, we succeeded in expression cloning of the cDNA encoding ET-1-selective receptor, ET-A receptor (ET-AR), from the bovine lung using the Xenopus oocyte expression system coupled with the voltage clamp method (9), while Sakurai et al. (10) reported the cDNA cloning of the non-isopeptide-selective ET receptor, ET-B receptor (ET-BR), from the rat lung using COS-7 cell expression system. Subsequently, several groups including ours have reported the cDNA clonings of rat and human ET-ARs (11-13) and human and bovine ET-BRs (14, 15). Cloning of a gene for any type of ETR in any species, however, has not been reported yet.

In order to establish the foundation for further analyses concerning possible genetic disorders of the ET-AR, we have now cloned and characterized the gene for the human ET-AR (hET-AR) and investigated the chromosomal location and the expression of the hET-AR gene.

EXPERIMENTAL PROCEDURES

Materials—Materials were obtained from the following sources: human genomic libraries from Clontech; pBluescript from Stratagene; restriction endonucleases from Toyobo; Sequenase from United States Biochemical Corp.; Superscript™ reverse transcriptase from Bethesda Research Laboratories; Gene Amp DNA amplification reagent kit and Taq polymerase from Perkin-Elmer Cetus Corp.; T4 polynucleotide kinase from Takara Shuzo; oligo(dT)-Latex from Nippon Roche; Colony/Plaque Screen from Du Pont; Biodyne nylon membrane from Pall Ultrafine Filtration Corp.; DNA of human-rodent somatic hybrid cell lines from Coriell Institute for Medical Research; cultured human umbilical vein endothelial cells from Colonnets Corp.; other reagents were described previously (9, 13).

Isolation and Characterization of Genomic Clones—Two human genomic libraries, one derived from leukocyte DNA in λEMBL4 and another derived from placenta DNA in λEMBL38P6/T7, were used in this study. Approximately 1 x 10⁶ recombinants were screened for each library using [³²P]-labeled full-length hET-AR cDNA probe (~470 to +3620) as previously described (16). The sequence of the hET-AR cDNA is numbered sequentially from the first nucleotide of the methionine initiation codon (Fig. 2). Four genomic clones were isolated from the two libraries and characterized by restriction endonuclease mapping. The restriction digests were subjected to electrophoresis, transferred to a nylon membrane, and were hybridized to the full-length hET-AR cDNA probe. All hybridizing genomic fragments were subcloned into pBluescript for further restriction analysis and sequencing. Intron sizes were determined by Southern blotting, restriction mapping, and the polymerase chain reaction (PCR) method (17).

DNA Sequencing—DNA sequencing was carried out by the di-
deoxy-chain termination method using Sequense. Sequence-specific oligonucleotides were synthesized using a 381A DNA synthesizer (Applied Biosystems). The strategy for sequencing is shown in Fig. 1.

Polymerase Chain Reaction—PCRs (17) were used to generate the hET-AR cDNA probe (+429 to +540) and the hET-AR cDNA probe (+1441 to +1808), to determine the sizes of introns or to confirm the transcription start site. PCRs were carried out using the Gene Amp DNA amplification reagent kit and Taq polymerase. The reaction was cycled 30 times in a cycle profile of 30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C. Amplified DNA fragments were analyzed by agarose gel electrophoresis.

Primer Extension—Primer extensions were carried out using two synthetic oligonucleotides, PE-1 (−408 to −437, 5′-GTCGGCTTCTCCCGCTTCTCCCTCCAAAGAAGGCGCTGCGTTGCTC-3′) and PE-2 (−390 to −415, 5′-CGGAGGAGAACAGGCTCCAGTCTGTCC-3′), under conditions described by McKnight and Kingsbury (18). The oligonucleotides were end-labeled with [γ-32P]ATP, hybridized to 10 μg of the human lung poly(A)+ RNA, and extended using Superscript™ reverse transcriptase. Fifty μg of total RNA from the human kidney cortex and liver were used as negative controls, since Northern blot hybridization analysis revealed that the hET-AR mRNA level was minimal in the kidney cortex (data not shown) and that hET-AR mRNA was absent in the liver (Fig. 8). The primer-extended products were separated on a 8 M urea, 5% polyacrylamide gel and then analyzed by autoradiography.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)—The human lung poly(A)+ RNA was primed using PE-3 (−365 to −394, 5′-AGGGCTCGGACGAAAAAGAAAACCTCGGGGAG-3′) as a specific primer for hET-AR mRNA. cDNA synthesis was accomplished with Superscript™ reverse transcriptase. Then the cDNA using 0.5 μg of poly(A)+ RNA was subjected to PCR, with a sense primer, PE-4 (−502 to −480, 5′-CCGGGCCTCGGAGTATCTGCTCGG-3′), or PE-5 (−521 to −503, 5′-GCTTTGTGCTTTTTAGTGG-3′) and an antisense primer, PE-6 (−406 to −423, 5′-GAGCTCCTGTCCTCCCGTCC-3′). The RT-PCR products were analyzed by 3.0% agarose gel electrophoresis with ethidium bromide staining.

Southern Blot Hybridization Analyses of the Human ET-AR Gene—Agarose gel-purified genomic DNA was digested with EcoRI, HindIII, and PstI, separated on a 0.7% agarose gel and then transferred to a nylon membrane. The membrane was hybridized with the 32P-labeled hET-AR cDNA probe (+429 to +540) as previously described (9, 13) and washed at 50 °C in 0.1 M NaCl, 5 mM sodium citrate, ph 7.0, 1% SDS.

The clones were hybridized with EcoRI-digested DNA of 24 human-roden somatic hybrid cell lines, as well as human and rodent parental cell lines. The chromosome content was determined by the supplier (Table II). Blots were hybridized with the 32P-labeled hET-AR cDNA probe (+1441 to +1808) in the 3′-noncoding region as previously described (9, 13) and then washed at 50 °C in 0.1× SSC, 0.1% SDS. Scoring was determined by the presence (+) or absence (−) of the human band in the hybrids on the blots.

Northern Blot Hybridization Analyses of Human ET-AR mRNA—Human tissues were obtained at autopsy or operation. Informed consent was obtained from each family. This study was approved by the ethical committee on human research of Kyoto University (No. 61-98). The human umbilical vein endothelial cells and mesangial cells were cultured as previously reported (19, 20). Northern blots of RNA on a nylon membrane were hybridized with 32P-labeled EcoRV- EcoRI fragment (+255 to +1080) of the hET-AR cDNA in a solution containing 50% formamide, 4× SSC, 5× Denhardt’s solution (1× Denhardt’s solution is 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 0.2% Ficol), 0.5% SDS, 10% dextran sulfate, and 250 μg/ml denatured salmon sperm DNA. The probe was labeled by random-primer synthesis to the specific activity of 2.5 × 106 cpm/μg DNA. The blots were washed at 60 °C in 0.1× SSC, 0.1% SDS. Autoradiography was performed for 3 days at −70 °C using Konica X-ray films with enhancing screens.

RESULTS

Isolation and Characterization of the Human ET-AR Gene—We screened overall 2 × 106 recombinants for two human genomic libraries, and λETAR1, λETAR2, and λETAR4 from human placenta genomic library. The four clones were mapped with restriction endonucleases (Fig. 1). The restriction fragments containing exons or the 5′-flanking region were subcloned and further mapped. There was no overlap between λETAR1 and λETAR2, and between λETAR2 and λETAR3, while λETAR3 and λETAR4 shared the same EcoRI site (Fig. 1). The nucleotide sequences of the exons, the exon-intron junctions, the 5′-flanking, and 3′-flanking regions were determined.

Structure of the Human ET-AR Gene—Comparison of the sequence of the hET-AR gene with that of the cDNA established the organization of the hET-AR gene (Fig. 1). The hET-AR gene spans more than 40 kilobases (kb) and consists of eight exons and seven introns. The exon and intron sizes and interrupted amino acids are shown in Table I. The GT-AG sequence is conserved for all splice sites (Fig. 2 and Table I) (21). Intron 1 occurs in the 5′-noncoding region. Exon 2 extends from 71 base pairs (bp) upstream of the ATG initiation codon to the nucleotide encoding the amino-terminal residue of the first extracellular loop. Exon 3 encodes the first extracellular loop and the third membrane-spanning domain. Exon 4 encodes the second intracellular loop, the fourth membrane-spanning domain, and the second extracellular loop. Exon 5 encodes the fifth membrane-spanning domain and the third intracellular loop. Exon 6 encodes the sixth membrane-spanning domain and the third extracellular loop. Exon 7 encodes the seventh membrane-spanning domain.

Analysis of the 5′-Flanking Sequence of the Human ET-AR Gene—To determine the transcription start site, we performed primer extension experiments using two primers, PE-1 (−408 to −437) and PE-2 (−390 to −415). In primer extension with the human lung poly(A)+ RNA, PE-1 gave a signal at −502 (Fig. 3A), while PE-2 revealed two signals at −502 and −499 (Fig. 3B). The stronger signal by PE-2 was detected at −502, corresponding to the signal revealed by PE-1. In primer extension with total RNA from the kidney cortex and liver as negative controls, neither PE-1 nor PE-2 gave a signal at −502, indicating the specificity of the signal of the lung poly(A)+ RNA. The transcription start site was then assigned to the cytosine residue 502 bp upstream of the methionine initiation codon.

To confirm the result of the primer extensions, we performed RT-PCR of the hET-AR transcript. Poly(A)+ RNA from the human lung was reverse-transcribed into cDNA with PE-3 (−365 to −394) as a specific primer for hET-AR mRNA. Then the cDNA was subjected to PCR. With a sense primer, PE-4 (−502 to −480) and an antisense primer, PE-6 (−406 to −423), a single DNA fragment of the expected size (97 bp) was amplified (Fig. 4), indicating that there is no intron between the sequences of PE-4 and PE-6. Detection of potentially containing genomic DNA was excluded, since no band was amplified with the human lung poly(A)+ RNA which had not been reverse-transcribed as a template (data not shown). By using the cDNA as a template, no DNA fragment with the expected size (116 bp) was amplified in the PCR with PE-5 (−521 to −503) and PE-6. The data of the RT-PCR were well consistent with the result of the primer extensions.

We sequenced the 5′-flanking region to 858 bp upstream of the transcription start site (Fig. 5). The 5′-flanking region lacks a typical TATA box (22) but contains a potential SP-1-binding site 27 bp upstream of the transcription start site.
**FIG. 1.** Schematic representation of the hET-AR gene and cDNA. a, the restriction map of the four genomic clones, \(\lambda hETAR_1\), \(\lambda hETAR_2\), \(\lambda hETAR_3\), and \(\lambda hETAR_4\). There is no overlap between \(\lambda hETAR_1\) and \(\lambda hETAR_2\) and between \(\lambda hETAR_2\) and \(\lambda hETAR_3\), while \(\lambda hETAR_3\) and \(\lambda hETAR_4\) share the same EcoRI site; B, BamHI; E, EcoRI. b, the strategy for sequencing. Exons are represented by closed boxes and are numbered. Arrows indicate the extent of sequence obtained. c, the diagram of the gene for the hET-AR. Exons are represented by closed boxes and are numbered. The restriction sites described in a are indicated by vertical lines. d, the structure of the hET-AR cDNA (18). The regions encoding the membrane-spanning domains are represented by open boxes and are numbered. The regions encoding the non-membrane-spanning domains are represented by closed boxes. The sites for translation initiation (ATG) and termination (TGA) are indicated.

| Exon (size) | 5' Splice donor | Intron (size) | 3' Splice acceptor | Exon |
|-------------|-----------------|---------------|--------------------|------|
| Exon 1      | TCCAGGtccg      | -72           | Inton 1 (~4.0 kb)  | -71  |
| (431 bp)    |                 |               | tcagGTGAAA        |      |
| Exon 2      | TTTAAGgtagg     | 420           | Inton 2 (~16 kb)  | 421  |
| (491 bp)    | PheLys          |               | tcagCTGCTG        | LeuLeu, 141 |
| Exon 3      | TGCAAGgtgaat    | 548           | Inton 3 (~11 kb)  | 549  |
| (128 bp)    | AspAr           |               | tcagGTACAG        | LeuLeu, 183 |
| Exon 4      | ATGGGGtagtact   | 747           | Inton 4 (~2.8 kb) | 747  |
| (199 bp)    | MetGlut         |               | tgagTTCTAC        | PheTyr, 250 |
| Exon 5      | AAGCAGggaaa     | 900           | Inton 5 (~3.2 kb) | 901  |
| (153 bp)    | LysGln          |               | tcagGTGCGA        | ArgArg, 301 |
| Exon 6      | ACTTAGgtag      | 1034          | Inton 6 (~0.43 kb)| 1035 |
| (154 bp)    | LeuSe           |               | tcagTTTTCTT       | rPheLeu, 345 |
| Exon 7      | TTCCAGGtgaag    | 1143          | Inton 7 (~1.0 kb) | 1144 |
| (109 bp)    | PheGln          |               | ccagGTACGC        | SerCys, 422 |
| Exon 8      |                 |               |                   |      |
| (2477 bp)   |                 |               |                   |      |

**TABLE I**

Exon-intron organization of the hET-AR gene

Exon sequences are in upper case letters; intron sequences are in lower case. The deduced amino acid sequence is shown below the nucleotide sequence.

A comparison of the 5' flanking sequence with established consensus sequences revealed the presence of a CArG box (23) at -1043, four GATA motifs (24, 25) at -1290, -1220, -1018 and -851, an octanucleotide sequence of CCATGTGC at -1163, which is an inverted sequence of the MyoD-E2A-binding site (26), and a hexanucleotide sequence of TCCAG at -1320, which is an inverted sequence of "the acute-phase reactant regulatory element" (27, 28) (Fig. 5).
Human Endothelin-A Receptor Gene

The nucleotide sequence of the hET-AR gene. The exon sequences are shown in upper case letters. The intron and the flanking sequences are in lower case. Nucleotides of the transcript are numbered sequentially from the first nucleotide of the methionine initiation codon. A CArG box, four GATA motifs, a potential SP-1-binding site, an inverted sequence of acute-phase reactant regulatory element, and an inverted sequence of MyoD-E2A-binding site are underlined. The polyadenylation signal is doubly underlined. Six GT clusters downstream of the polyadenylation site are underlined with dashes. The deduced amino acid sequence is shown above the nucleotide sequence. Amino acids are numbered sequentially from the translation initiation site. Positions of the putative membrane-spanning domains I-VI are indicated above the sequence.

Analysis of the 3'-Flanking Sequence of the Human ET-AR Gene—There is a potential polyadenylation signal 22 bp upstream of the polyadenylation site of the cDNA, which is followed by six GT clusters (Fig. 2). These two features have been reported to represent conserved areas for transcription termination and 3'-processing (29).

Southern Blot Hybridization Analyses of the Human ET-AR Gene—To determine the number of genes encoding the
**Human Endothelin-A Receptor Gene**

A. PE-1

\[
\begin{align*}
123 & T G C A \\
\end{align*}
\]

B. PE-2

\[
\begin{align*}
1 & 2 T G C A \\
\end{align*}
\]

**FIG. 3.** Primer extensions. Primer extensions using oligonucleotides, PE-1 (−408 to −437) (panel A), and PE-2 (−390 to −415) (panel B). The end-labeled primers were hybridized to 10 µg of poly(A)^+ RNA from the human lung and extended with Superscript™ reverse transcriptase (panel A, lane 1; panel B, lane 2). Fifty µg of total RNA from the human kidney cortex (panel A, lane 2) and liver (panel A, lane 3; panel B, lane 2) were used as negative controls. The primer-extended products were separated on an 8 M urea, 5% polyacrylamide gel. PE-1 gave a signal at −502, while PE-2 revealed two signals at −502 and −499, as indicated by the arrows. Marker lanes T, G, C, and A indicate sequencing ladders of the hET-AR gene using the same primers.

**FIG. 5.** Nucleotide sequence of the 5′-flanking region of the hET-AR gene. The transcription start site is indicated by an inverted triangle. A CArG box, four GATA motifs, a potential SP-1-binding site, an inverted sequence of acute-phase reactant regulatory element (APRRE), and an inverted sequence of MyoD-E2A-binding site are enclosed by boxes.

**FIG. 6.** Southern blot hybridization analyses of the hET-AR gene. 10 µg of human genomic DNA digested with EcoRI, HindIII, and PstI were probed with the hET-AR cDNA probe (+429 to +540). Sizes in kb are indicated on the left. For chromosome 4 (Table II), indicating that the hET-AR gene was assigned to chromosome 4.

**Gene Expression of the ET-AR in Human Tissues**—We extensively investigated the gene expression of the ET-AR in human tissues using Northern blot hybridization analysis method with the EcoRV-EcoRI fragment of the hET-AR cDNA (+225 to +1080) as a probe (Fig. 8). Human ET-AR mRNA was detected as a single band with a size of 4.3 kb at the highest abundance in the aorta, at high levels in the lung, atrium, colon, decidua, chorion, and amnion and at moderate levels in the cerebral cortex, cerebellum, ventricle, kidney, adrenal, and duodenum. No hybridizing signal was detected for chromosome 4 (Table II), indicating that the hET-AR gene was assigned to chromosome 4.
Human Endothelin-A Receptor Gene

FIG. 7. Hybridization of DNA from human-rodent somatic hybrid cells and rodent parental cells with hET-AR cDNA probe. Southern blots were prepared from a human-hamster somatic hybrid cell (NA10115) (lane 1), a human-mouse somatic hybrid cell (NA10478) (lane 2), a hamster parental cell (NA10658) (lane 3), and a mouse parental cell (NA05862) (lane 4). 90% of NA10115 cells had a human chromosome 4. 8% of NA10478 cells had a human chromosome 4, while 84% contained a human chromosome 20 (Table II). The blots were probed with the hET-AR cDNA probe (+1441 to +1808) in the 3'-noncoding region. No band was detected in hamster and mouse parental cells. A single discrete signal was detected in the NA10115 cells with a size of 7 kb, which was consistent with the restriction map (Fig. 1). A faint band with the same size was observed in the NA10478 cells (lane 2).

in the liver. A moderate level of ET-AR mRNA was demonstrated in the cultured human mesangial cells, while no band was detected in the cultured human endothelial cells of the umbilical vein.

DISCUSSION

In the present study we have clarified the organization, structure, chromosomal assignment, and expression of the hET-AR gene. The present study demonstrates that the hET-AR gene contains introns 2–7 in the coding region (Fig. 1). Genes for many members of the G protein-coupled receptor superfamily, such as the β-adrenergic receptor (30), were reported to contain no intron in the coding region, but subsequent studies have revealed that, among genes for G protein-coupled receptors, the genes for the substance K receptor (SKR) (31), substance P receptor (SPR) (32, 33), neurenomed K receptor (NKR) (33), dopamine D2, D3, and D4 receptors (D2R, D3R, and D4R) (34–36), opsins (37), and luteinizing hormone receptor (LHR) (38), contain introns in the coding region (Fig. 9). The ET-AR gene elucidated in the present study belongs to the latter group. As shown in Fig. 9, exon/intron splice sites of the G protein-coupled receptors are compared. The genes for the ET-AR, SKR, SPR, NKR, D2R, and D4R have an intron at the same location immediately after the third membrane-spanning domain. This finding may suggest that the genes for these receptors originated from the same ancestral intron-containing gene. Another finding of the exon-intron organization of the hET-AR gene is that intron 2–7 in the coding region occurs before or after the regions encoding the membrane-spanning domains (Fig. 1). This finding suggests that each exon encodes a potential functional unit of the receptor.

The present study also demonstrates that intron 1 occurs in the 5'-noncoding region. To date, among genes for G protein-coupled receptors, only the gene encoding the M2 muscarinic acetylcholine receptor has been discovered to contain an intron in the 5'-noncoding region (39). The M2 muscarinic receptor cDNAs have been reported to contain multiple alternatively spliced 5'-noncoding regions. However, we have not isolated any hET-AR cDNA with alternatively spliced 5'-noncoding regions from the human placenta cDNA library (13).

With the primer extension experiments, we demonstrated that the transcription start site is located 502 bp upstream of the methionine initiation codon (Fig. 3). The results of the RT-PCR are consistent with those of the primer extensions (Fig. 4). We sequenced the 5'-flanking region to 858 bp upstream of the transcription start site. The 5'-flanking region lacks a typical TATA box but contains a potential SP-1-binding site 27 bp upstream of the transcription start site (Fig. 5). A comparison of the 5'-flanking sequence with established consensus sequences reveals the presence of a CARG box, four GATA motifs, an inverted sequence of the MyoD-E2A-binding site, and an inverted sequence of the acute-phase reactant regulatory element. These findings will facilitate the elucidation of the regulation of the gene expression.

On the basis of human-rodent somatic hybrid cell DNA, we demonstrated that the hET-AR gene is localized to human chromosome 4 (Table II). Further studies on subchromosomal localization of the hET-AR gene using in situ hybridization techniques may allow for identification of closely linked genes or genetic disorders.

The present study demonstrates that hET-AR mRNA is expressed in a wide variety of human tissues (Fig. 8). In the previous study we have demonstrated that ET-BR mRNA is expressed in a wide variety of human tissues, at high levels in the cerebral cortex and cerebellum, and at moderate levels in the placenta, lung, kidney, adrenal, colon, and duodenum (14). Thus, the distribution of hET-AR mRNA overlaps with that of hET-BR mRNA. The rank order of the mRNA levels, however, is different between ET-AR and ET-BR. The present study demonstrates the highest level of ET-AR mRNA in the human aorta and lack of hybridizing signal in the cultured human endothelial cells. By contrast, in the previous study we detected significant levels of ET-BR mRNA in the human aorta and cultured human endothelial cells (14). These findings indicate that the major subtype of ETR in vascular smooth muscle cells is ET-AR and that the predominant subtype in vascular endothelial cells is ET-BR. The ET-AR in vascular smooth muscle cells may mediate ET-1-induced vasorelaxation, while the ET-BR in vascular endothelial cells may play a role in the release of prostacyclin and endothelium-derived relaxing factor (40). The present study demonstrates that the gene expression of the ET-AR is distributed in a wide variety of human tissues including non-vascular tissues (Fig. 8). Since the gene expression of ET isopeptides was detected in a wide variety of human vascular and non-vascular tissues by Yanagisawa, the ET-AR in non-vascular tissues may mediate the paracrine control of ET isopeptides. Further studies are required for the elucidation of biological functions of the ET-AR in non-vascular tissues.

In the present study we demonstrate a substantial level of hET-AR mRNA in the cultured human mesangial cells (Fig. 8). Since the origin of mesangial cells is considered to be the same as that of vascular smooth muscle cells, this finding is

2 M. Yanagisawa, personal communication.
Segregation of the ET-AR with human chromosomes in EcoRI-digested DNA from human-rodent somatic hybrid cell lines

The hET-AR cDNA probe (+1441 to +1808) was hybridized to Southern blots prepared from human-rodent somatic hybrid cell DNA. The chromosome content was determined by the supplier. Scoring was determined by the presence (+) or absence (−) of human band in the hybrids on the blots. Concordant hybrids have either retained or lost the human band together with a specific human chromosome. Discordant hybrids have either retained the human band but not a specific chromosome, or the reverse. Percent discordancy indicates the degree of discordant segregation for a marker and a chromosome. A 0% discordance is the basis for chromosome assignment.

| DNA No. | hybrid | ET-AR | Percentage of cells with human chromosomes |
|---------|--------|-------|------------------------------------------|
| 1       | 2      | 3     | 4  5  6  7  8  9  10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 |
| G54.207299 | -      | 25  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.210826B | -      | 0  96 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010153 | -      | 0  0 100 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010115 | +      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010114 | +      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.10101629 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.10101790 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010156B | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010161 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010926B | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.10109278 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010686 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010986 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010579 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010146 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010567 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010496 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.10101010 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010649 | +      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010678B | +      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010737 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1010899 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.1081086 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |
| G54.10636317 | -      | 0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0  0 |

* A faint band was observed in the Southern blot analysis (Fig. 7).

Fig. 8. Northern blot hybridization analyses of hET-AR mRNA. 20 μg of total RNA from the human aorta, lung, atrium, ventricle, cerebral cortex, cerebellum, liver, kidney, adrenal, duodenum, colon, deciducta, chorion, amnion, cultured human mesangial cells, and cultured human endothelial cells of the umbilical vein were used. The presence of intact 28S ribosomal RNA (rRNA) was confirmed in all lanes (not shown). The positions of 28S and 18S rRNA are shown on the left.

consistent with our notion that the ET-AR is the major subtype of ETR in vascular smooth muscle cells. The cultured human mesangial cells will be a useful tool for the investigation of the regulation of the expression of the hET-AR gene.

The increase of ET-1 receptor has been reported in ischemic myocardium, the kidney injured by cyclosporine, and the brain in spontaneously hypertensive rats (41). The present study should give a clue to the discovery of possible disorders of the ET-AR, as well as facilitate the elucidation of the mechanisms by which the gene is regulated.

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