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Identification of a Novel Gene with RING-H2 Finger Motif Induced after Chronic Antidepressant Treatment in Rat Brain

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Previously, we have identified 200 cDNA fragments as antidepressant related genes/ESTs. In this study, using these cDNAs, we developed our original cDNA microarray for rapid secondary screening of candidate genes as the novel therapeutic targets. With this microarray, we found that the expression of a novel gene, ADRG34, was significantly increased in rat hippocampus which had been chronically treated with a selective serotonin reuptake inhibitor antidepressant, sertraline. RT-PCR analysis also demonstrated the induction of ADRG34 at mRNA levels in rat hippocampus and the frontal cortex. This cDNA encoded 685 amino acid residues containing a RING-H2 finger motif at the carboxy-terminal. Sequence analysis of ADRG34 with the EMBL/GenBank database showed significant homology to mouse and human kf-1 gene. Our data suggest that ADRG34, a possible rat homologue of kf-1, may be one of the common functional molecules induced after chronic antidepressant treatment.

Key Words: **SSRI; sertraline; depression; cDNA microarray; differential display PCR.**

Antidepressants have been used clinically since 1950's. It has been demonstrated that many antidepressants acutely inhibit monoamine transporters, resulting in significant increase in synaptic concentrations of monoamines, noradrenaline or serotonin. However, there is a latency period of several weeks before the onset of clinical effect of antidepressants. Hyman and Nestler proposed a paradigm, initiation and adaptation, within which to conceptualize the drug-induced neural plasticity that underlies the long-term actions of antidepressants in the brain (1). However, the detailed mechanisms underlying drug-induced adaptive neuronal changes are not known. The therapeutic action of antidepressants could be the results of indirect regulation of other neuronal signal transduction systems or their changes at the molecular level by an action on gene transcription induced after chronic treatment. Indeed, there are selective effects of antidepressants on specific immediate early genes and transcription factors including, c-fos (2, 3), zif268 (2), NGFI-A (4, 5), Arc: activity regulated cytoskeleton associated protein (6) and the phosphorylation of CRE binding protein (7). These molecules would be important for adaptive neuronal changes after chronic antidepressant treatment. Previously, region specific effect of chronic antidepressant treatment on the DNA-binding activities for CRE-, SP1-, and GRE-binding elements were reported in rat hippocampus and frontal cortex (8). Alterations in functional proteins that are related to the neural plasticity, PKC and GAP-43, in the brain of depressed suicide victims are also reported (9). Together, these data may demonstrate the possible role of changes in gene expression in the mechanism of antidepressant action.

Recent developments in molecular neurobiology provide new conceptual and experimental tools for understanding the mechanisms by which antidepressant produce long lasting alterations in brain function. With RNA fingerprinting technique, a modified differential display PCR, we had been continuing our effort to elucidate the involvement of some common biochemical changes induced after chronic treatment with two different classes of antidepressants, imipramine (a tricy-

The GenBank accession number for the nucleotide sequence is AF306394.

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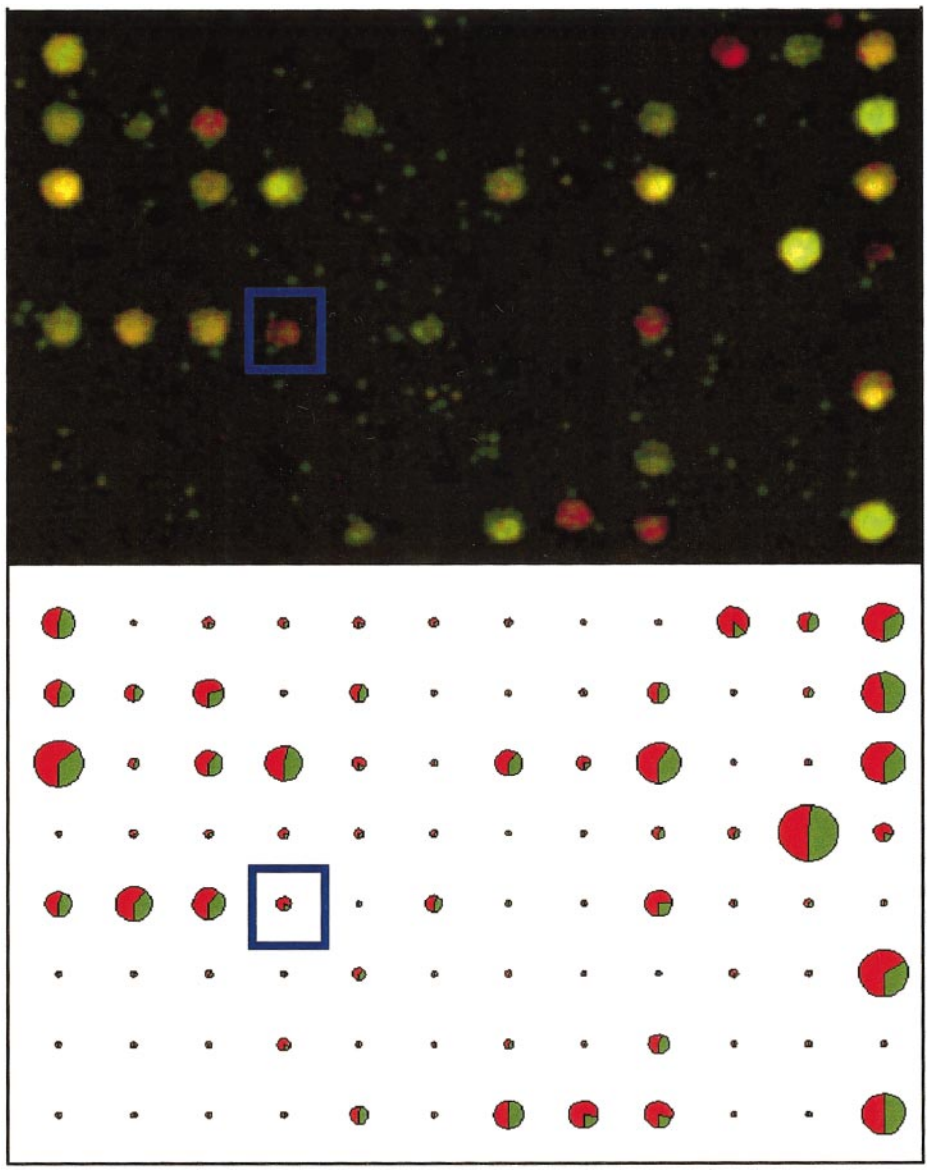

FIG. 1. The pseudo-color image of ADRG microarray after hybridization with fluorescence probes. Ninety-six spots representing ADRG1-96 (top) and the analyzed data using ImaGene software (bottom) are shown here. The pseudo-color images of control group data (green) and sertraline group (red) were overlapped. The spot with the blue rectangle represents ADRG34. Although the expression levels were relatively low, the fluorescence intensities for ADRG34 were 3.91 times increased in sertraline group when compared to controls.

clic antidepressant) or sertraline (a serotonin selective reuptake inhibitor, SSRI). Until now, we have molecularly cloned 200 cDNA fragments as expressed sequence tags (ESTs), which we named them antidepressant related genes, ADRG1-200. One of the ADRG genes was identified as HSC49, a novel splice variant of HSC70 by our group previously (10). More recently introduced technique, cDNA microarray, is an efficient method to perform large-scale coordinate monitoring of gene expression during different functional states in normal and diseased samples, or in control and treated animals. In addition to the gene expression monitoring, cDNA microarray can be used for gene discovery by probing enriched libraries derived from the experiments with differential cloning techniques. In this study, to find molecular machineries which is responsible for the therapeutic action of antidepressant, we developed our original cDNA microarray (ADRG microarray) using ADRG genes derived from our previous experiments with RNA fingerprinting.

By gene expression analysis using ADRG microarray and fluorescence-labeled probes, we identified several interesting candidate genes and ESTs. One of the spot, ADRG34, was significantly increased in sertraline treated rat hippocampus on the ADRG microarray. Homology analysis of ADRG34 with the EMBL/

TABLE 1

The Expression of kf-1 after Chronic Antidepressant Treatments

Brain region	Control	Imipramine	Sertraline
Hippocampus	100 ± 3.7	204.0 ± 20.8*	173.4 ± 13.6*
Frontal cortex	100 ± 9.3	165.6 ± 9.9*	182.2 ± 8.8*
Hypothalamus	100 ± 18.8	112.9 ± 8.9	129.2 ± 14.6

Note. Data are expressed as % of the control data (means ± SEM) of five independent experiments.

* $P < 0.05$, Student's t test.

GeneBank database showed significant matches to mouse and human kf-1 gene (11). Kf-1 was originally identified as the gene whose expression has been augmented in the cerebral cortex of a sporadic Alzheimer's disease patient (11). Here, we first report the induction of a newly cloned gene, ADRG34, after chronic antidepressant treatment in rat brain.

MATERIALS AND METHODS

Experimental animals. Male Sprague-Dawley rats (age 7–10 weeks, Sankyo Labo Service Co., Tokyo, Japan) were housed in a temperature controlled environment with 12 h light/12 h dark cycle with free access to food and water. Rats were randomly separated into control and treated groups. Experimental animals for chronic treatment of antidepressants received either vehicle for 21 days, 5 mg/kg of imipramine (Sigma Chemical Co., St. Louis, MO) or sertraline (Pfizer Pharmaceuticals Inc., NY), dissolved in 1.5% tween 80, by daily intraperitoneal injection. Six rats were used for each treatment group. Animals were killed by decapitation, and brain was quickly removed, dissected and then frozen in liquid nitrogen immediately and stored at −80°C until use. All studies using animals were carried out in accordance with animal protocols approved by the National Institutes of Health.

RNA fingerprinting. RNA fingerprinting was done as described by our group previously (10). Total RNA from rat frontal cortex was extracted by Isogen reagent (Nippon Gene Co., Tokyo, Japan) following the manufacturer's instruction. Isolated total RNA was then dissolved in RNase-free water and the concentration was estimated by UV spectrometry. Total RNA samples were treated with RNase-free DNase I for 30 min at 37°C, purified by phenol-chloroform extraction, and used for RNA fingerprinting. RNA fingerprinting study was then carried out in the presence of [^{33}P]dATP (Life Science Products, Inc., Boston, MA) using mRNA fingerprinting kit (Clontech, Palo Alto, CA) following the manufacturer's instructions. Radio-labeled PCR products were then analyzed by electrophoresis on denaturing 6% polyacrylamide gels. Three individual samples of each treatment were applied side-by-side and visualized by autoradiogram.

Subcloning and sequence analysis. The bands of our interest were cut out from dried gel and the cDNA fragments were extracted, and then reamplified by the same primer set used for RNA fingerprinting. The PCR conditions were 94°C for 3 min followed by 40 cycles of 94°C denaturing for 30 s, 60°C annealing for 1 min, and 72°C extension for 1 min. Reamplified product was ligated into pCR II-TOPO vector (Invitrogen, Carlsbad, CA) and transformed into competent TOP 10F' *Escherichia coli* cells (Invitrogen, Carlsbad, CA). Sequence analysis was performed by dideoxy sequencing methods. Homology search and sequence alignment was done using the FASTA search servers at the National Center for Biotechnology Information. Additional cDNA sequence information of 5′ and 3′ end of ADRG34 was obtained by RACE PCR (rapid amplification of cDNA ends PCR) using primer sequences derived from RNA fingerprinting.

Fabrication of cDNA microarray and fluorescence image analysis. To develop ADRG microarray for the secondary screening of candidate genes, each of the ADRG 1–200 cDNA inserts were amplified by vector primers and spotted in duplicated on the glass slide using GMS417 Arrayer (Genetic MicroSystems Inc., Woburn, MA) with the modified method of Salunga (12). In addition, negative controls (a plasmid vector DNA) and ten different kinds of positive controls, so called house keeping genes, were also spotted on the same glass slide for normalization. To make the fluorescence-labeled probe for hybridization, total RNA samples obtained from rat hippocampus from control or sertraline group was extracted by Isogen reagent (Nippon Gene Co., Tokyo, Japan) following the manufacturer's instruction. Then, three independent total RNA samples from each group were pooled and used for the next procedure. Poly A^+ RNA was then purified from pooled total RNA with oligo-dT columns (Takara, Tokyo, Japan). One microgram of poly A^+ RNA from control or sertraline samples was converted to cDNA in the presence of Cy-5 or Cy-3-dUTP respectively to make fluorescence-labeled probes. Hybridization of probes to microarray was done competitively. The probes were mixed and placed on an array, overlaid with coverslip, and hybridized for 16.5 h at 65°C. After hybridization and washing procedure, each slide was scanned with GMS418 Array Scanner (Genetic MicroSystems Inc., Woburn, MA). Then, gene expression levels were quantified and analyzed using ImaGene software (BioDiscovery Ltd. Swansea, UK).

Northern blot analysis. Complimentary DNA fragment of ADRG34 obtained from RNA fingerprinting was cut out from PCR II-TOPO vector and labeled with [α-^{32}P]dCTP, and then used as a probe. Rat multiple tissue Northern blot nylon membrane (Clontech, Palo Alto, CA) was used for the experiment. Hybridization procedure was carried out following the manufacturer's instructions. After the hybridization, the membrane was exposed to X-ray firm for 24 h.

Messenger RNA expression analysis with RT-PCR. The first strand cDNA was synthesized with reverse transcriptase and 1 μM of oligo-dT primer, from 2 μg of total RNA samples treated with RNase-free DNase I, and diluted to a final volume of 100 μL. One microliter of each cDNA sample was added to 24 μL of PCR reaction mixture containing 0.5 μM of a pair of primers for ADRG34, 5′-GGAATACGGACAGGACTTTC-3′ and 5′-TCCGAGAAGCTGCATGGGC-3′ (Amersham Pharmacia Biotech, Tokyo, Japan). A pair of primers for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH), a housekeeping gene, 5′-TGAAGGTCGGTGTCAACGGATTTGGC-3′ and 5′-CATGTAGGCCATGAGGTCCACCAC-3′ were also used for normalization. To ensure the fidelity of this analysis, we assayed several cycles of PCR to determine the liner range for amplification of PCR product in each region of the brain. Amplification of ADRG34 was performed as follows: 3 min at 94°C for initial denaturation, 25 cycles (hippocampus), 23 cycles (frontal cortex) or 26 cycles (hypothalamus) of 94°C denaturing for 30 s, 55°C annealing for 30 s, and 72°C extension for 1 min, followed by a final extension at 72°C for 7 min. Amplification of GAPDH was performed as follows: 3 min at 94°C for initial denaturation, 25 cycles (hippocampus), 16 cycles (frontal cortex) or 22 cycles (hypothalamus) of 94°C denaturing for 30 s, 55°C annealing for 30 s, and 72°C extension for 1 min, followed by a final extension at 72°C for 7 min. The PCR products were electrophoresed in a 1% agarose gel containing SYBR green, a nucleic acid gel stain reagent GelStar (Takara, Tokyo, Japan). The optical density of the digitized image was quantified using a fluorescence image analyzer, FM-bio II (Hitachi, Tokyo, Japan).

RESULTS AND DISCUSSION

Identification of quantitative changes in gene expression that occur in the brain after chronic antide-

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   1 cagctgcagc gggtccagtg ctccgggggc gacggccgtg gcctgcagag gcggcaggga
  61 cggtggccgc ggttggcgcg cgcatccgca cggggctagt ccccgcgcag ctacgcaact
 121 gacaggaagc ctccctggag agccgcgtcg gggcctagtg ttatttgctt tttgcttttt
 181 ctccccttc acgactgtgg tctcgcgctc ttccgcagcg ggagccgccg cgacgccccc
 241 tcgcgggccg ccggcctgag aggcgccgcc cgcgggaacc tggagccgcc gccgggcctg
 301 ggcggccgcc ggggcctgaa gcctgggcgt tcggcgcggc gctgcgacga ccgctccaac
 361 ccgcgctggg cgcgccgggc cccaggcctg gcccagccga gcaccgcgcc ttccggactg
 421 gggccacgta gtccggcgcc aaccgccctc cgctggcccg cagtctatcg gagctgcctc
 481 ctggtcaccc ctggtgggct cttgccctct tccagccttc cgcagttcga tgggtggagt
 541 gcttttgaac gtgccctctt ctccctttct cagctcttga ccgcaaggcc agagccgggg
 601 ccctcagccg cacgcacgca gcgatccacc tgcctccccg cggggatggc ccgcgggtgc
 661 cgccaccgcc gcgcgccctc gctgccctcg ccgccctcgc caggctacgc tcgtcgcccc
 721 gcactcgacc ccctcttctt gtccacggcg tcccggctcc cggcgacgcc aagagggcga
 781 agATGTGGCT GAAGCTGTTT TTCTTGCTCC TGTATTTCCT GGTCCTGTTC GTCCTGGCCA
 841 GGTTTTTTGA GGCCATTGTG TGGTACGAGA CTGGCATCTT TGCTACTCAG CTGGTGGATC
 901 CGGTGGCATT GAGCTTCAAG AAGCTGAAGA CCATTCTGGA GTGTCGAGGG CTGGGCTACT
 961 CCGGACTACC TGAGAAGAAA GATGTACGGG AGCTGGTGGA GAAGTCAGGT GACTTGATGG
1021 AAGGTGAACT CTATTCTGCT CTCAAGGAAG AAGAAGCATC TGAGTCTGTT TCTAGTACCA
1081 ATTTCAGTGG TGAAATGCAT TTCTATGAGC TTGTAGAAGA CACAAAAGAT GGCATCTGGC
1141 TGGTTCAGGT CATAGCAAAT GACAGAAGTC CTTTGGTGGG TAAAATCCAC TGGGAGAAAA
1201 TGGTAAAAAA AGTGTCAAGA TTTGGAATAC GGACAGGCAC TTTCAACTGT TCCAGTGATC
1261 CCAGGTACTG CAGAAGGAGA GGCTGGGTAC GTTCCACTCT CATCATGTCT GTCCCACAAA
1321 CAAGCACATC TAAAGGGAAA GTCATGCTTA AAGAGTACAG TGGGCGCAAG ATTGAAGTAG
1381 AACACATTTT TAAATGGATA ACTGCCCATG CAGCTTCTCG GATCAAAACT ATATATAATG
1441 TTGAGCATTT GAAAGAAGAA TGGAATAAAA GTGATCAGTA CTGGGTAAAA ATATACCTGT
1501 TTGCAAACCT TGACCAACCA CCAGCTTTCT TCTCTGCATT AAGTATAAAA TTTACTGGAA
1561 GAGTTGAGTT TATTTTTGTT AATGTGGAAA ATTGGAACAA CAAGAGTTAT ATGACAGATA
1621 TTGGTATTTA TAACATGCCG TCATACATAC TTAGAACTCC TGAAGGAATT TACAGATATG
1681 GAAATCACAC AGGTGAATTT ATATCCCTTC AGGCCATGGA TTCATTTTTA CGCTCATTAC
1741 AACCTGAAGT AAATGATCTG TTTGTTTTGA GTTTGGTTCT AGTTAATCTT ATGGCTTGGA
1801 TGGACTTATT TATTACACAA GGAGCAACCA TCAAGCGATT TGTGGTTCTC ATAAGCACTT
1861 TAGGGACATA CAATTCCCTA TTAATTATTT CTTGGCTACC TGTGTTGGGC TTTCTACAGC
1921 TCCCTTACTT AGATAGCTTT TATGAATATA GTTTAAGATT GCTGCGATAC TCTAATACAA
1981 CCACACTGGC TTCGTGGGTA AGGGCAGACT GGATGTTTTA CTCTTCACAC CCAGCCCTGT
2041 TTCTCAGTAC ATACCTTGGA CATGGTTTGC TAATTGATTA CTTTGAGAAG AAGAGACGGC
2101 GCAGCAACAA TGATGAAGTT AATGCGAATA ATTTAGAGTG GTTATCAAGT CTGTGGGACT
2161 GGTACACCAG CTACCTCTTC CACCCGATTG CTTCTTTTCA GAACTTTCCT GTAGACTCTG
2221 ATTGGGATGA AGACCCTGAC TTATTCTTGG AGCGGTTAGC TTTCCCTGAC CTTTGGCTTC
2281 ACCCTCTGAT ACCAACTGAT TATATTAAAA ACTTACCAAT GTGGCGGTTT AAATGTCTTG
2341 GGGCTCAGTC TGAAGAAGAA ATGTCGGAGA GTTCTCAAGA CACTGAAAAT GACTCAGATA
2401 GTGACAACAC GGACACTTTT AGTAGTAGTA AGGATGTATT TGAAGATAAA CAAAATGTTC
2461 ACAGTTCTCC AGGAAGAACA AGTCGCTGCG ATACTGAGGC TTGTTCATGT GCCAATAAAT
2521 GTGTCAGCAG CCCATGTGAA AGGAAGAGGA GGTCATATGG CTCACATAAT ACTAAGGAAG
2581 ATATGGAGCC GGACTGGCTA ACTTGGCCTG CTGGTACGCT GCACTGTACT GAATGTGTTG
2641 TTTGCCTTGA GAATTTTGAG AATGGATGTT TGCTGATGGG GTTGCCTTGT GGTCATGTGT
2701 TTCACCAGAA TTGCATTGTT ATGTGGTTGG CTGGGGGCCG ACACTGTTGC CCTGTTTGCC
2761 GTTGGCCTTC ATATAAGAAA AAGCAGCCAT ATGCACAGCA ACAGCCGTTG TCAAATGATG
2821 CTCCATCTtg accatgtgca agttgtccaa taagctttga gtatcttaca gcttgccttt
2881 ttaatgttag tcacaatgtt tttgtggttt gaagtttagt ttaatgttag tgcagtgaca
2941 ggaaatacac attatgctga tgttgatgac agaatttatt tggatgcctt gtgtgtcaat
3001 tgaatgcata ctaaactgta aaaaaattat ttacagcatt gaaaattcag aagttaatgg
3061 ttttttgtaa gcacaaaaga agtatggtag aaatttatct tagcaagact ttatgaggca
3121 ggatcaaatc ctagtgggcc tgagctgatt tcttacccta aatgtttttt ccctttttac
3181 aatctctgtc cagcacctct tggttaaata atgtatgctc tgagacatga aattaaaaca
3241 gatctataaa ataaattatt ttaaaagcaa aaaaaaaaaa aaaaaaa
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FIG. 2. Nucleotide sequence of ADRG34. The nucleotide positions are numbered on the left. The open reading frame (783–2828) is capitalized and underlined. The cDNA fragment obtained from RNA fingerprinting (992–1528) is double underlined.

pressant treatment can yield novel molecular markers that may be useful in the diagnosis and treatment of major depression. Using differential cloning strategy, we and other groups have reported the isolation of some genes that are differentially expressed in the brain after chronic antidepressant treatment (10, 13, 14).

In the present study, we identified several interesting candidate genes and ESTs by gene expression analysis using ADRG microarray and fluorescence-labeled

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ADRG34      MWLKLFFLLL YFLVLFVLAR FFEAIVWYET GIFATQLVDP VALSFKKLKT   50
mouse kf-1  .......... .......... .......... .......... ..........   50
human kf-1  .......... .......... .......... .......... ..........   50

ADRG34      ILECRGLGYS GLPEKKDVRE LVEKSGDLME GELYSALKEE EASESVSSTN  100
mouse kf-1  .......... .........V .......... .......... ..........  100
human kf-1  .......... .........E .......... .......... ..........  100

ADRG34      FSGEMHFYEL VEDTKDGIWL VQVIANDRSP LVGKIHWEKM VKKVSRFGIR  150
mouse kf-1  .......... .......... .......... .......... ..........  150
human kf-1  .......... .......... .......... .......... ..........  150

ADRG34      TGTFNCSSDP RYCRRRGWVR STLIMSVPQT STSKGKVMLK EYSGRKIEVE  200
mouse kf-1  .......... .......... .......... .......... ..........  200
human kf-1  .......... .......... .......... .......... ..........  200

ADRG34      HIFKWITAHA ASRIKTIYNV EHLKEEWNKS DQYWVKIYLF ANLDQPPAFF  250
mouse kf-1  .......... .........V .......... ....V..... ..........  250
human kf-1  .......... .........A .......... ....L..... ..........  250

ADRG34      SALSIKFTGR VEFIFVNVEN WNNKSYMTDI GIYNMPSYIL RTPEGIYRYG  300
mouse kf-1  .......... .......... .N........ .......... ..........  300
human kf-1  .......... .......... .D........ .......... ..........  300

ADRG34      NHTGEFISLQ AMDSFLRSLQ PEVNDLFVLS LVLVNLMAWM DLFITQGATI  350
mouse kf-1  .......... .......... .......... .......... ..........  350
human kf-1  .......... .......... .......... .......... ..........  350

ADRG34      KRFVVLISTL GTYNSLLIIS WLPVLGFLQL PYLDSFYEYS LRLLRYSNTT  400
mouse kf-1  .......... .......... .......... .......... .R........  400
human kf-1  .......... .......... .......... .......... .K........  400

ADRG34      TLASWVRADW MFYSSHPALF LSTYLGHGLL IDYFEKKRRR -SNNDEVNAN  449
mouse kf-1  .......... .......... .......... .......... -S........  449
human kf-1  .......... .......... .......... .......... NN........  450

ADRG34      NLEWLSSLWD WYTSYLFHPI ASFQNFPVDS DWDEDPDLFL ERLAFPDLWL  499
mouse kf-1  .......... .......... ........D. .......... ..........  499
human kf-1  .......... .......... ........E. .......... ..........  500

ADRG34      HPLIPTDYIK NLPMWRFKCL GAQSEEEMSE SSQDTENDSD SDNTDTFSSS  549
mouse kf-1  .......... .......... .V........ S........D .D.M..F..S  549
human kf-1  .......... .......... .V........ G........E .E.T..L..E  550

ADRG34      KDVFEDKQNV -.SSPGRTSR CDTEACSCAN K-CVSSPCER KRRSYGSHNT  597
mouse kf-1  .DI....... V.S...RT.H ..T....... .-.ES..... .R.....H..  598
human kf-1  .EV....... L.N...TA.H ..A....... .Y.QT..... .G.....Y..  600

ADRG34      KEDMEPDWLT WPAGTLHCTE CVVCLENFEN GCLLMGLPCG HVFHQNCIVM  657
mouse kf-1  D......... ...GT..... .......... .......... ..........  658
human kf-1  N......... ...DM..... .......... .......... ..........  650

ADRG34      WLAGGRHCCP VCRWPSYKKK QPYAQQQPLS NDAPS  682
mouse kf-1  .......... .......... .....Q.... ..V..  683
human kf-1  .......... .......... .....H.... ..V..  685
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FIG. 3. Alignment of the deduced amino acid sequence of ADRG34 and mouse and human kf-1 proteins. The amino acid positions are numbered on the right. The RING-H2 finger motif is double underlined.

probes. The pseudo-color image of ADRG microarray after hybridization is shown in Fig. 1. As expected, we obtained low background and consistent results in duplicated experiments. After normalization with the signals for both negative and positive controls, several spots of our interest on the ADRG microarray showed increased or decreased fluorescence intensities after chronic sertraline treatment (data not shown). Interestingly, although the expression levels were relatively low, the fluorescence intensities for a newly cloned gene, ADRG34, was 3.91 times increased in sertraline group when compared to controls. The induction of ADRG34 after chronic antidepressant treatment was also confirmed by RT-PCR analysis. The reproducible band corresponding to ADRG34 at the size of 199 bp existed on a gel. As shown in Table 1, we have demon-

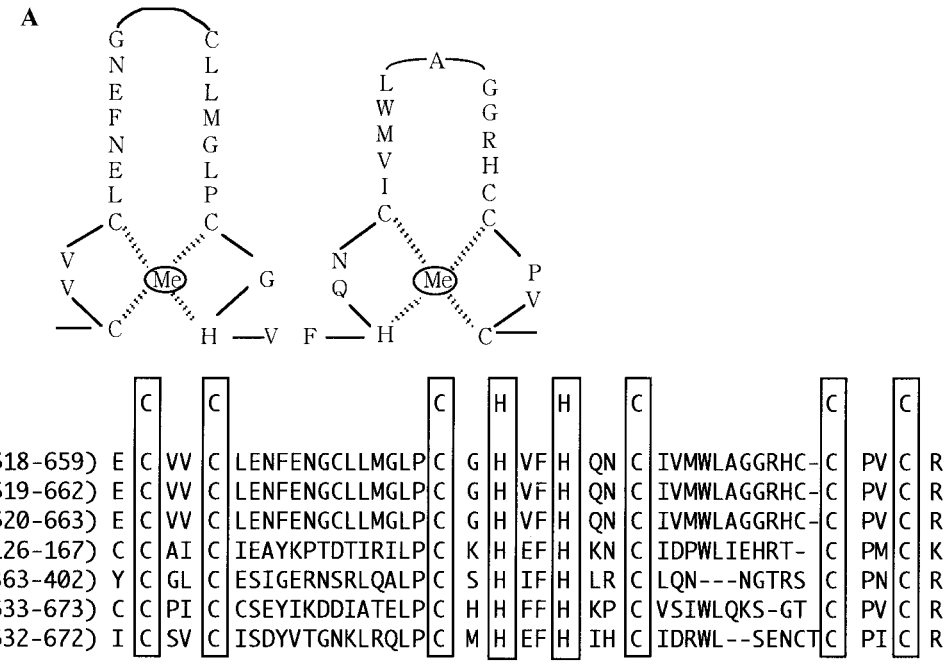

FIG. 4. The putative secondary structure of RING-H2 finger domain of ADRG34 (A) and predicted amino acid alignment of RING-H2 finger motif of various proteins (B). Conserved cysteine and histidine in RING-H2 finger motif could bind to divalent metal (Me) ions (A). The amino acid sequences of RING-H2 finger family proteins were obtained from EMBL/GenBank. Genbank accession numbers are *Drosophila* goliath (M97204), mouse 43-kDa receptor-associated protein of synapse, RAPsyn (J03692), rat Neurodap1 (D32249), and human RNF6 (AJ010346). Boxes indicated the conserved cysteine and histidine residues of the RING-H2 finger motif (B).

strated that the treatment with imipramine or sertraline induced the expression of ADRG34 at mRNA levels in rat hippocampus (204 ± 20.8% or 173.4 ± 13.6%, respectively) after normalization by GAPDH expression. The hippocampus is one of the several brain regions that would be involved in the endocrine, emotional, cognitive, and vegetative abnormalities found in depressed patients. Interestingly, it is demonstrated that chronic stress causes atrophy of hippocampal neurons and that the volume of hippocampus is decreased in depressed patients (15, 16). Hippocampus has been associated with learning and memory and therefore the induction of ADRG34 could also be involved in some therapeutic effects on cognitive functions. Hippocampus is also involved in feedback regulation of the hypothalamus-pituitary-adrenal axis, and depression is associated with dysfunction of this neuroendocrine axis (17). However, the mRNA level of ADRG34 was not significantly changed in the hypothalamus (Table 1). On the other hand, the expression of ADRG34 was also increased in antidepressant treated group in rat frontal cortex (165.6 ± 9.9% or 182.2 ± 8.8%, respectively). The frontal cortex is another region of the brain which is implicated in the pathophysiology of depression. In the frontal cortex, glucose metabolism, blood flow, and electroencephalograph (EEG) activity are altered in depressed patients (18). It is reported that NGFI-A mRNA expression was increased in the hippocampus and in the cerebral cortex after antidepressant treatment (4, 5). Thus, NGFI-A may be a mediator of ADRG34 induction after antidepressant treatments. Although it is possible that the therapeutic action on a single brain region underlies antidepressant treatment, it is also possible that pharmacological effects on multiple brain regions contribute the real therapeutic action of antidepressants. Studies to further characterize the neuronal circuitry of these brain regions will help elucidate the neuroanatomical substrates of antidepressive effects.

In this study, we have determined the nucleotide sequence of the full length cDNA for ADRG34 (Fig. 2). The 537-bp cDNA fragment originally obtained from RNA fingerprinting (992–1528) is double underlined. Additional cDNA sequence information of 5′ and 3′ end of ADRG34 was obtained by 3′- and 5′-RACE PCR. Northern blot analysis demonstrated the presence of a single transcript of about 3.5 kb in size for mRNA prepared from several rat tissue regions, which hybridized to the [^{32}P]-labeled ADRG34 probe. These regions included brain, lung and kidney, liver and heart, but at much lower levels in spleen and muscle (Fig. 5). The open reading frame (783–2828) is highlighted in Fig. 2. This cDNA encoded 685 amino acid residues yielding a mass of 79 kDa, containing a RING-H2 finger motif at the carboxy-terminus (Figs. 3 and 4A).

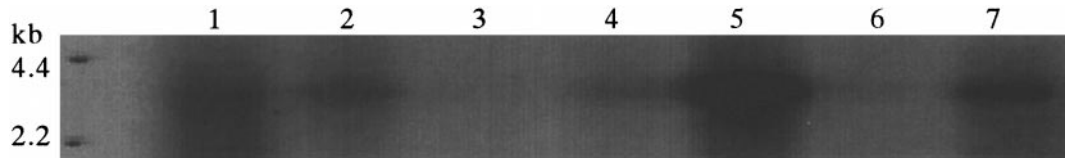

FIG. 5. Northern blot analysis of ADRG34 in rat tissues. Complimentary DNA fragment of ADRG34 obtained from RNA fingerprinting was labeled with [α-^{32}P]dCTP and used as a probe. Rat tissues (1, heart; 2, brain; 3, spleen; 4, lung; 5, liver; 6, muscle; 7, kidney) was analyzed by Northern blot. There is a single transcript of about 3.5 kb.

Sequence analysis of ADRG34 with the EMBL/GenBank database showed very high homology (95.2 and 81.9% at nucleotide levels and 98.5 and 95.5% at deduced amino acid levels, respectively) to mouse and human kf-1 gene (11). Alignment of the deduced amino acid sequence of ADRG34 and mouse and human kf-1 proteins is shown in Fig. 3. Kf-1 was originally identified as the gene with RING-H2 finger motif whose expression have been augmented in the cerebral cortex of a sporadic Alzheimer's disease patient. Subsequently, mouse kf-1 was identified by the same group. The RING-H2 finger motif of ADRG34 was identical to those of mouse and human kf-1 (Fig. 4B). RING-finger motif is a subclass of zinc finger motif, found in the sequence of the human ring 1 gene (19). The RING-H2-finger motif is closely related to the RING-finger motif, in which the fourth cysteine is replaced by a histidine; Cys-X(2)-Cys-X(12-35)-Cys-X-His-X(2)-His-X(2)-Cys-X(8-39)-Cys-X(2)-Cys, where X refers to an arbitrary amino acid residue (20). Amino acid sequence of the carboxy-terminal region of Kf-1 was compared with other RING-H2 finger family proteins, *Drosophila* goliath (21), mouse 43-kDa receptor associated protein of synapse, RAPsyn (22), rat Neurodap1 (23) and human RNF6 (24) (Fig. 4B). The metal binding ligands were perfectly matched between these family members. This comparison indicated that the carboxy-terminal domain of Kf-1 has the same structure as defined by the RING-H2 finger motif. The various proteins with RING-H2 finger motif shown in Fig. 4B have diverse functions. Goliath has been implicated to play a developmental role in mesoderm formation or differentiation (21). RAPsyn was reported to be involved with the clustering and aggregation of acetylcholine receptors (22). Neurodap1 has been shown to mediate synaptic communication and plasticity through the control of the formation of postsynaptic density for maintaining vital functions of nerve cells (23). RNF6 was cloned and mapped close to the chromosome 13 breakpoint in a case of myelofibrosis with a t(4;13)(q26;q12) (24). The precise physiological function of ADRG34 protein is as yet unclear, though current evidence suggests that it may be involved in protein-protein interactions and play a role in the assembly of large multiprotein complex (25). On the other hand, we have previously reported that the expression of 49 kDa of heat-shock cognate protein (HSC49), a novel splice variant of HSC70, was increased by chronic antidepressant treatment (10). Heat-shock protein family are a ubiquitous and abundant family of molecular chaperons involved in a wide range of cellular processes, such as assembly/disassembly of multimetric complexes (26), including those of the glucocorticoid receptor (27) and heat shock transcription factor (28). Although, the relationship between coordinated upregulation of HSC49 and ADRG34 by chronic antidepressant treatment is still unclear, protein-protein interactions may be related to the therapeutic action of antidepressant. Many of the previous reports have focused on synaptic pharmacology, especially on neurotransmitter turnover and neurotransmitter receptors. To understand the therapeutic actions of antidepressants, we must now extend its efforts beyond the synapse, to an understanding of cellular and molecular neurobiology as well as to a better understanding of the architecture and function of neural systems.

In conclusion, we demonstrated here that ADRG34, a possible rat homologue of mouse and human kf-1, is one of the common functional molecules induced after chronic antidepressant treatment, and may be associated with the mechanism of action of various antidepressant treatments in the alleviation of depression.

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