Differentially Expressed Genes in the Nucleus Accumbens from Chronically Ethanol-Administered Rat

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The isolation of differentially expressed genes in the nucleus accumbens (NA) from chronically ethanol-administered rats may help in understanding the underlying molecular mechanisms for the development and reinforcement of ethanol addiction. The differential display indicated that around 0.1–0.2% of mRNA could be considered to be affected by chronic ethanol-administration in the NA, regardless of whether ethanol directly affected gene expression in the NA or the gene alteration was secondary to changes in neuronal activity caused by ethanol. Forty-six clones were successfully reamplified, and screening by reverse Northern blot analysis resulted in the isolation of five up-regulated and three down-regulated genes. One of the up-regulated cDNAs was homologous to human TGFβ1 and its preferential expression was also observed in the cerebellum and locus coeruleus (LC). Since clone c10 displayed an extremely strong preferential expression in the ethanol-administered NA, its upstream sequence was analyzed by 5′ rapid amplification of DNA ends (5′RACE) but the coding sequence has not yet been isolated. c118 showed enrichment in the ethanol-administered NA and displayed strong homology to the mouse KH domain RNA binding protein QKI-5A. The 5′RACE analysis confirmed that this clone encoded rat QKI-5A. Since QKI proteins are considered to be regulators of myelination and their absence causes dysmyelination, its up-regulation may offer protection against ethanol-induced dysmyelination. Another 12 cDNAs were registered as expression sequence tags (ESTs) or novel with their functions unknown. It is considered important, however, that to their upstream sequences including coding regions and promoter sequences are identified not only to estimate the roles of these differentially expressed genes in ethanol addiction but also to clarify whether ethanol-dependent gene-regulation can occur or not.

Key words —— chronic ethanol administration, differential display, rat, nucleus accumben

INTRODUCTION

Alterations in neuronal membrane fluidity are thought to be related to tolerance for and dependence on ethanol.[1,2] Recently, the importance of the basal ganglia/limbic striatal and thalamocortical circuits, the brain reward systems, has been emphasized in terms of craving and loss of control in ethanol abuse and dependence as well as of other psychotropic drugs such as cocaine and morphine, *etc*.[3–6] However, the roles of the various brain regions involved in these circuits seem to depend on the drug species since progression of dependence differs from drug to drug. A number of studies indicated that the nucleus accumbens (NA) plays an important role in progression of chronic ethanol addiction.[6–9] The locus coeruleus (LC) also seems to be involved in this progression because long-term ethanol use can cause an increase in local cerebral glucose utilization in the LC as well as the NA[10] but its importance is especially recognized in morphine tolerance.[4,5]

The experiments using alcohol-preferring rats[7] suggest that the serotonergic pathway from the dorsal raphe nucleus to the NA mediates the reinforcing actions of ethanol. Among serotonin receptor subtypes, the serotonine 3 receptor is considered to be responsible for such actions.[8] The importance of dopaminergic projection in the striatoacumbal-

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ventral pallidal neuronal circuits has also be pointed out.[3,11] In addition to serotonin and dopamine, multiple transmitter systems in the brain reward systems, such as GABA, glutamate, and opioids are thought to be involved in ethanol reinforcement.[9,12]

In addition to direct cytotoxicity, not only ethanol but also enhanced generation of its metabolite, acetaldehyde, may cause secondary metabolic disturbances, enzyme inactivation, a reduction in DNA repair and so on.[13] Although it is still controversial whether ethanol and/or acetaldehyde directly affect gene regulation, the least that can be said is that the cAMP and phosphoinositide signal-transdution pathways may be the targets that mediate the action of ethanol and contribute to the molecular events involved in the development of ethanol tolerance.[14] Ethanol can activate several subtypes of adenylate cyclase when its stimulatory G protein is activated and cause an increase in cAMP levels *in vitro*,[13] hence it is conceivable that ethanol has at least some effect on cAMP responsive element binding protein (CREB)-related genes.[14] The catalysts of ethanol, *i.e.* alcohol dehydrogenase and aldehyde dehydrogenase, are themselves known to be induced by ethanol administration.[15] In the brain reward systems, subjecting rats to chronic ethanol exposure induces up-regulation of the *N*-methyl-D-aspartate receptor R1 subunit in the hippocampus,[16] and of dopamine 3 receptors in the limbic forebrain including the NA,[17] while it causes a reduction in dopamine transporters and an increase in cFos of dopaminergic neurons in the NA.[8]

Identification of differentially expressed genes in a specific brain region may help not only to understand the underlying molecular mechanisms for development and reinforcement of ethanol addiction but also to clarify whether ethanol-dependent gene-regulation occurs. In the study presented here, differential display was used to isolate differentially expressed genes in chronically ethanol-administered NA for a comparison with control ones.

MATERIALS AND METHODS

Ethanol Administration and Total RNA Purification —— Male Wister rats (150–170 g body weight) were treated with an ethanol-containing liquid diet: For the first week, animals were given a nutritionally adequate liquid diet containing (percentage of total calories) 5% fat, 18% protein, and 77% carbohydrates *ad labium*.[18] Pair feeding was then begun, with 3% ethanol given on the first 2 days, 4% ethanol on days 3 and 4, and 5% ethanol (36% of calories) for the duration of the treatment (28 days). Control rats received an isocaloric amount of carbohydrate instead of ethanol. Thereafter, total RNA was isolated from the NA, cerebellum, and LC of both control and ethanol-administered rats by using the guanidium-isothiocyanate and cesium chloride method.[19]

Differential Display —— Pairs of control and ethanol-administered mRNAs from the NA, cerebellum, and LC were simultaneously analyzed using differential displays.[20] Total RNA (2.5 μg) was reverse transcribed in 20 μl of an RT buffer containing 0.2 μM of anchored polydT primer 5′-AAGCT11V-3′ (GeneHunter Corp., U.S.A.), 100 μM of dNTPs, and 1 U of MMuLV reverse transcriptase (GeneHunter Corp., U.S.A.). After reverse transcription, aliquots were diluted with 80 μl of TE[19] and 10 μl aliquots were stored at –80°C until use. Two μl of the cDNA solution was then subjected to PCR amplification with 10 μM of each anchored primer and one of the arbitrary primers, AP49-56 (GeneHunter Corp., U.S.A.) in 50 μl of a PCR buffer containing 100 μM of dNTPs, 0.25 μl of α-^{32}P-dCTP (110 Tbq/mmol, Amersham Pharmacia Biotech, U.K.), and 1U of *Taq* polymerase (Takara, Japan). Two cycles of PCR were performed at 94°C for 2 min, at 40°C for 5 min, and at 72°C for 5 min for 2 cycles, and for 28 cycles at 94°C for 30 sec, at 55°C for 30 sec, and at 72°C for 1 min. Amplified ^{32}P-labeled cDNA was electrophoresed on 6% denaturing acrylamide gels, which were exposed to X-ray films overnight. The bands showing different intensities in the control/ethanol-administered comparison or the comparison among brain tissues were excised from the gels, soaked in 50 μl TE[19] and boiled for 15 min. The cDNA fragments were then PCR re-amplified and subcloned into a pGEM-T vector (Promega, U.S.A.). The resultant clones were sequenced with a dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems, Japan) and analyzed with the aid of the GenBank BLAST and FAST homology search programs. The cloned cDNAs were also subjected to reverse Northern blot analysis.

Reverse Northern Blot Analysis —— Reamplified cDNAs were blotted on a nitrocellulose membrane. First-strand cDNA was synthesized from RNAs of the control/ethanol-administered NA as described earlier, in the presence of α-^{32}P-dCTP. ^{32}P-labeled cDNA was used as a probe and hybridized to the

membrane in HB-N[19] at 42°C overnight. The membrane was washed twice with 2 × SSC and 0.1% SDS at room temperature and twice with 0.5 × SSC and 0.1% SDS at 65°C for 15 min. After drying, the membrane was exposed to a BAS-III imaging plate (Fujix, Japan) and image analyzed with a BAS 2000 image analyzer (Fujix, Japan).

5′ Rapid Amplification of DNA Ends (RACE) —— 5′RACE used Marathon™ cDNA amplification kit (Clontech, U.S.A.) according to the manufacturer's protocol. That is, poly(A)$^+$RNA was purified from total RNA obtained from the ethanol-administered NA and subjected to double strand cDNA synthesis. After ligation with the cDNA adapters, the cDNAs were PCR amplified with a combination of an adaptor primer, AP1, and a synthetic oligomer specific to isolated cDNA. The obtained products were reamplified by nested PCR using a set of a nested adapter primer, AP2, and a synthetic nested oligomer specific to isolated cDNA. The reamplified products were then cloned and sequence analyzed as described earlier.

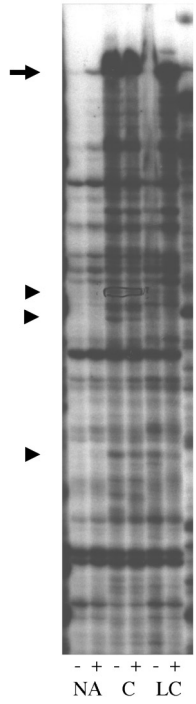

Fig. 1. Representative Data of Differential Display

A set consisting of an arbitrary primer, AP50, and an anchored polydT primer, 5′-AAGCT11A-3′, was used. The arrow and arrow heads rindicate differential expression in, respectively, control amd ethanol-administered rats and regionally differential expression.

RESULTS

Differential Display

Not only the control/ethanol-administered comparison but also the regional comparison could be made when differential display PCR products from control/ethanol-administered NA, cerebellum, and LC were simultaneously electrophoresed (Fig. 1). Each product could be separated into more than 300 bands on a denaturing acrylamide gel. A comparison of the intensity of each band demonstrated that a number of bands displayed the same intensity in any region whether they were from control rats or ethanol-administered ones. However, in addition to the region-specific expression (Fig. 1, arrow heads), there were cDNAs enriched in either control or ethanol-administered rats (Fig. 1 arrows). Of the 150 cDNAs excised from the gels, 50 were selected and PCR-reamplified. Since around 80% of the cDNAs could be reamplified and cloned, 43 clones could be isolated (Table 1).

Identification of Isolated Clones

Three of the isolated clones included cDNA inserts of known proteins, like aldehyde reductase (c1), proto-oncogene Ret (c3), and glutamate dehydrogenase (c8) (Table 1). Clones, c7, c18, c21, c117, and c118 included inserts whose sequence was homologous to the mRNAs of other animal species. Twenty-two clones were registered as expression sequence tags (ESTs) whose coding proteins have not yet been identified and 12 clones were novel.

Differential Expression in Control/Ethanol-Administered NA

Although 27 were selected because they showed different expressions in the NA when the control/ethanol-administration comparison was performed, differential expression was confirmed in only six clones, *i.e.* four up-regulated and two down-regulated ones, as indicated by the results of reverse Northern blot analysis (Fig. 2 and Table 1). Up-regulation of c18 which is homologous to human TGFβ1 mRNA, was confirmed. The differential display showed that this mRNA was up-regulated in all the region examined. Another 16 clones were selected because of their differential expression in the cerebellum and LC but the reverse Northern blot analysis confirmed that one clone was up-regulated and one down-regulated in the ethanol-administered NA. Clones c10 and c118 in particular displayed a marked difference in expression between control and ethanol-administered NAs, so that they were subjected to 5′RACE analysis.

Table 1. Differentially Expressed Genes in the Nucleus Accumbens, Cerebellum, and Locus Coeruleus of Control/Ethanol-Administered Rats

Arbitrary primer	Anchor of polydT	No.	Gene name	Nucleus accumbens		Cerebellum		Locus coeruleus		Reference (bp)	Reverse Northern[d]	
				Control	Ethanol	Control	Ethanol	Control	Ethanol		Control	Ethanol
49	A	1	Aldehyde reductase	−	+	−	−	−	+	163	*c*)	*c*)
		2	EST206613	−	+	−	−	−	+	390	*c*)	*c*)
		3	receptor tyrosine kinase (Ret gene)	+	++	+	±	+	++	360	+	+
	C	5	EST225559	−	++	−	−	−	−	390	*c*)	*c*)
		29	AA220782	−	++	+	+	+	+	341	+	++
	G	8	Gultamate dehydrogenase	++	+++	*b*)	*b*)	*b*)	*b*)	469	++	++
		9	EST235634	+	+++	*b*)	*b*)	*b*)	*b*)	512	++	++
		10	*a*)(+EST228122)	+	++++	*b*)	*b*)	*b*)	*b*)	421	−	+++
		12	human clone 24658	−	+	+	−	−	−	117	++	*c*)
		32	*a*)	−	+	+	+	−	+	260	+	+
		103	EST206613	+++	+	++	++	+++	+++	389	±	±
		104	EST206613	+	+++	+	+++	+	+++	390	+	+
		106	*a*)	+	+++	+	+++	−	−	210	+++	+++
		109	*a*)	−	+++	+	++	+	+	133	±	±
50	A	36	EST206613	−	+	−	−	−	−	389	*c*)	*c*)
		37	EST54211	+	+++	−	−	++	+	83	±	±
		38	EST202486	+	−	++	+	+	+	58	*c*)	*c*)
	C	15	AI072992	−	+	−	−	−	−	104	++	++
		17	human EST(AA174108)	+	−	+	−	+	+	104	+	+
		18	human TGF beta-1	−	+	++	+++	−	+	106	++	+++
		40	EST210417	−	+++	−	−	+	+	160	++	−
54	C	65	AI502391	−	++	*b*)	*b*)	*b*)	*b*)	334	+	+
55	A	117	Human CAGF-9 mRNA	−	+	−	+	−	−	144	+	+
55	C	51	*a*)	−	++	*b*)	*b*)	*b*)	*b*)	404	±	±
		56	EST227005 Rat embryo	+	+++	−	−	−	++	115	*c*)	*c*)
		57	*a*)	+	++	+	++	+	++	110	++	+
55	G	122	*a*)	−	++	−	−	−	−	271	+	++
49	A	26	*a*)	−	−	+	++	*b*)	*b*)	510	+	+
	G	7	human FLJ23250 fis	+	+	+	−	+	+	450	+	+
50	C	39	EST206613/EST218538	−	−	++	−	−	−	188	*c*)	*c*)
		21	human p62 mRNA	−	−	++	++	−	−	242	++	++
		22	*a*)	−	−	++	++	−	−	126	+	+
		24	EST206613/EST225559	−	−	+++	++	+	−	397	++	++
51	C	25	EST206613/EST225559	−	−	+	−	+	−	391	+	+
		49	*a*)	++	++	−	−	−	−	73	++	++
54	C	67	AI228864	+	+	+++	+++	++	−	320	+	+
55	A	59	EST219758	−	−	+	+++	−	−	221	+	+
		118	mouse KH domain RNA binding protein QKI-5A	+	+	+	+	+++	+++	103	+	++
		119	EST224532	+	+	−	+	+	+	104	±	±
55	C	52	*a*)	−	−	−	+	−	−	191	+	+
56	C	123	BF550457	−	−	−	+	−	+	181	++	+
56	G	101	EST224532	*b*)	*b*)	*b*)	*b*)	+	−	296	*c*)	*c*)

a) not registered, *b*) could not be elongated, and *c*) could not be estimated. *d*) cDNA synthesized from mRNA of control/ethanol-administered nucleus accumbens was used as a probe.

5′RACE Analysis of Clones c10 and c118

The insert of clone c10 included a poly(A) tail as well as a poly(A) signal so that at first it was thought to be the 3′ terminal of a novel mRNA (Fig. 3A). Based on the result of the 5′RACE analysis, this cDNA was registered as EST228122. How-

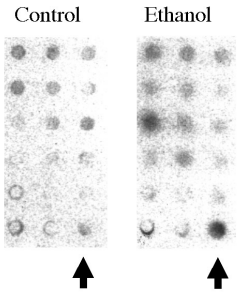

Fig. 2. Representative Data of Reverse Northern Blot Analysis
^{32}P-labeled cDNA synthesized from mRNA of either control or ethanol-administered NA was probed. Arrows indicate c10.

ever, EST228122 covers only 529 bs upstream to clone c10. Although further 5′RACE analysis could isolate more than 1500 bps of its upstream region, the coding region itself has not yet been confirmed. As for c118, only part of the coding sequence could be isolated. Sequence analysis, however, confirmed that this mRNA codes the rat KH domain RNA binding protein QKI-5A because its sequence displayed, respectively, 95 and 99% homology to human and mouse QKI-5A mRNA sequences (Fig. 3B).

A. upstream sequence of c10

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CCCCAAATCTGCAACCCCTACCTAAAAGCATAGGACTTAGGCTATGGATGCCTTCAAACCCGGCATGCGCAAATTTACACACATACGTCC
 P  Q  I  C  N  P  Y  L  K  A  *  P  K  S  A  T  P  T  *  P  N  L  Q  P  L  P  K  S  I  G

CTAGGTGCTTGTTTAGTGACACAACCTGTTGAAAAGGCAAGCTTCACGGTCAGTCAGAGACAGGGTCTCAAAAAATATATAGGAAAACAG
 L  R  L  W  M  P  S  N  P  A  C  A  N  L  H  T  Y  V  P  R  C  L  F  S  D  T  T  C  *

CAGGGGAGGGCACGGAGGGTTCTGACTTCTGCGTGTGCACAGATAGGTGTGCACACCAGACTTCTGCGTATACACTCATAGGTGTACACA

CCTGTATATACACAGACACACGCTTAGTGTGTCAAATTTCCAACCAGTTCAGTCTATTGACAGAATTCTATAGGAATCAATGTGTGATTT

CATCCACTCAGCTCACATTTATGAGGTGTGTGATGTTGTGTATTGATCTAGGCGTGATTGGAGAACCGTAGTGTGTACATACATGTCATA

GAGTGCACATACATGTCATAGAGTGCACATACACGTCATAGTGTGTACATACACGACATAGTGTGCACATGCATGTGTTTGATAGTCTAT

AGAACTGTGACACTGCATTTCAGACACTGACATTTTTTAAAAGACAATAATAAAACCCATCTTCATAATTGTGATTTCTCATGATGGGGT

CGTGGGAGTGAAATCCCTTCCTCTCTCCGTCTAGGTGTTCATCTACATCATCGTGTCTCCACTGTGCGGTGGCTTCACGTGGCCAAGCTG

TGTGAAGAAATAAAGGAAAAAAATCACTGTTCACCAAGGATGTGAGCATGGAAGGAGGAAAAGCCAATGCGTGTGGCCCTGGCTTTCACT

TCTCTCAGCAAACGCCATTTCTTTCATCCTCTTTCCTTTTTCTCTTTGCCCATCACACGGTTTGTGTTAACGAATTTCACATCGAGTCGT

TGTGCACAGGAGAAAGGACATCGTTTGTTCCTCCGATTTGTTAAGTAGAAGCTTCTAGAAAGACATCTTTGCTTCTAACATGCAAAGCTT

TTGCCAAAGTGGAAACGGCTGCGCTCACGACCTGTCTGTGAACTCACGACCTGTCTGCTAAACCTGTCTGTGACGTTGTGTGTTGACACA

AAGCACTGGGAAACGGGTTGCACCGCTGACATCTCACTTTAGTCTCTTTCGTTGTGGGAGAATTTTGAGTGCTTCACAATAATGGAGAAA

TTTGGGAATTATAATAAATTACAATAAATGGTCGGTGCTGCTTATGTCNCAGGAGCCCATCCGGGTAACGGTTAACGTTGAATTTTATCT

CAGTGAGGTGGGAAATGCCTTCAATACCTCAAGGACCATGGGTTAGCTGGATCCTATGGTCCAATGGGTAGAGCAGCCTGACGGCAGCCC

AGACCTGTTGAAGAGCCGTGGGTTCCAGGCCCCACACAAGCGAGCATTTTGATTGACAGACCATTAGAAGCACAAGGATGACCCTTCCTT

CTCCCAGCTTTGGCTTTCTGAAATGAAGTCCCGGACATTATTTATTCACTCTGCACATAGTTTAACACTTTCTGTTTATCCATTACCAAT

TCGAACTGAGATTAAGTAAAGGATNGGGGTTGGGGATTTAGCTCAGTGGTAGAGCGCTTGCCTAGGAAGCGCAAGGCCCTGGGTTCGGTC

CCCAGCTCCGAAATAAAAGAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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B. upstream sequence of c118

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TTTGTTGGGAGAATCCTTGGACCTAGAGGACTTACAGCTAAACAACTTGAAGCAGAAACGGGATGTAAAATAATGGTCCGAGGCAAAGGC
 F  V  G  R  I  L  G  P  R  G  L  T  A  K  Q  L  E  A  E  T  G  C  K  I  M  V  R  G  K  G

TCAATGAGGGATAAAAAGAAGGAGGAGCAAAATAGAGGCAAGCCCAATTGGGAGCATCTAAATGAAGACTTACATGTACTAATCACTGTG
 S  M  R  D  K  K  K  E  E  Q  N  R  G  K  P  N  W  E  H  L  N  E  D  L  H  V  L  I  T  V

GAAGATGCTCAGAACAGAGCAGAAATCAAGCTGAAGAGAGCGGTTGAAGAAGTGAAGAAGTTACTGGTACCTGCGGCTGAAGGTGAAGAC
 E  D  A  Q  N  R  A  E  I  K  L  K  R  A  V  E  E  V  K  K  L  L  V  P  A  A  E  G  E  D

AGCCTGAAGAAGATGCAGCTGATGGAGCTTGCAATTCTGAATGGCACCTACAGAGACGCCAACATTAAATCACCAGCCCTTGCCTTTTCT
 S  L  K  K  M  Q  L  M  E  L  A  I  L  N  G  T  Y  R  D  A  N  I  K  S  P  A  L  A  F  S

CTTGCAGCAACTGCCCAGGCTGCTCCAAGGATCATCACTGGGCCTGCGCCTGTCCTCCCACCAGCTGCTCTGCGTACACCTACGCCAGCT
 L  A  A  T  A  Q  A  A  P  R  I  I  T  G  P  A  P  V  L  P  P  A  A  L  R  T  P  T  P  A

GGCCCTACCATAATGCCTTTGATCAGACAAATACAGACCGCTGTCATGCCAAACGGAACTCCTCACCCAACTGCTGCAATAGTCCCTCCA
 G  P  T  I  M  P  L  I  R  Q  I  Q  T  A  V  M  P  N  G  T  P  H  P  T  A  A  I  V  P  P

GGGCCTGAAGCTGGGTTAATCTACACACCCTATGAATACCCCTACACATTGGCACCAGCTACATCAATCCTTGAGA----------
 G  P  E  A  G  L  I  Y  T  P  Y  E  Y  P  Y  T  L  A  P  A  T  S  I  L  E

CACTTCTGGGTTTTAGTTGGGATTTTACATAGCTTGCATTTAATTCTTTAGTTCTTTGCTGTTTCTATTAACCCATAGCATTATTTTAAT

AAATGTTATAATACCAACCTACTAAAAAAAAAAA
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Fig. 3. Upstream Sequence of c10 and c118 Obtained by 5′RACE Analysis
The deduced amino acid sequence is indicated by single letter symbols. Asterisks indicate stop codons. cDNA obtained by differential display PCR is underlined. A putative poly(A) signal was hatched. The sequence corresponding to EST228122 was underlined with a dotted line. (DDBJ accession No; AB054997 and AB054998).

DISCUSSION

When the annealing temperature was maintained as low as 37–40°C, only 4–6 bases of the 3′ terminal of primers could be annealed to the templates.[21] Thus, the arbitrary primer could identify any cDNA with a probability of $1/4^4$ to$1/4^6$, or 1/256–1/4056, so that cDNAs with different size could be assured to have originated from different mRNAs. Differential display PCR usually could amplify cDNAs shorter than 500–550 bps. That is, a set of one of the three anchored poly dT primers 5′-AAGCT11V-3′ and an arbitrary primer made it possible to compare around 400 different cDNAs. Since each cell was assured to contain 10^5–10^6 molecules of mRNAs regardless of their species, each lane of the differential display represented at least 0.04% of all mRNAs. In our study, eight different arbitrary primers, AP49–56, were applied to three anchored anchored poly dT primers so that this differential display could screen around 10000 cDNAs, *i.e*., around 1% of all mRNAs. From the 50 cDNAs selected from the 150 cDNAs excised from the gels, a total of eight cDNAs were isolated as a differentially expressed gene from the control/ethanol-administered NA. Thus, around 0.1–0.2% of the mRNA was thought to be affected by chronic ethanol-administration in the NA regardless of whether ethanol directly affected gene expression in the NA or the gene alteration was secondary to alteration in neuronal activity caused by ethanol.

Differential display is thus useful for isolating differentially expressed genes in more than two cellular populations,[22–24] and is an aid for the isolation of not only known mRNAs with specific functions for their originating cells, but also novel mRNAs whose function is not yet clear. The conventional radiolabeling differential display used in our study is extremely sensitive but, different from subtraction hybridization,[5,25] includes many false-positives, lack of 3′ end representation and redundancy between cDNA subsets. In fact, of the 27 clones which were selected because of differential expression in control and ethanol-administered NAs during differential display, the differential expression of only six (22%) was confirmed by reverse Northern blot analysis. On the other hand, of the 16 clones which were not expected to display differential expression in the NA, two were proven to show differential expression in control and ethanol-administered NAs. Since reverse Northern blot analysis is convenient and especially useful when the amount of mRNA is limited, but is sometimes less sensitive than the differential display, it should be noted that the results of reverse Northern included a number of false negatives. For example, aldehyde reductase and glutamate dehydrogenase were selected as up-regulated genes in the ethanol-administered NA but they were not always enriched in the reverse Northern blot analysis. However, these enzymes were related to energy metabolism and thus possibly affected by ethanol administration. In such a case, further examination will be required to confirm whether ethanol administration actually does not affect their gene regulation. However this may be, it is important to avoid false positives as well as false negatives when a control/ethanol-administered comparison is performed simultaneously for several brain regions.

Among the clones whose up-regulation was confirmed by reverse Northern blot analysis, c18, homologous to human TGFβ1, was up-regulated in all regions examined. The physiological function of TGFβs is still controversial but is thought to have at least three major effects: growth inhibition of epithelial, endothelial, and hematopoietic cells, stimulation of extracellular matrix formation, and immunosuppression.[26] Up-regulated TGFβ1 may thus exaggerate neuronal degeneration by chronic ethanol administration. The open reading frame of c10 has not yet been isolated so that its function remains unknown, although its mRNA was extremely enriched in the ethanol-administered NA. EST228122 was isolated from rat embryo but its regional distribution has not yet been examined. Not only isolation of its coding sequence but also identification of its regional expression patterns may help an understanding of its physiological function both in the presence and absence of ethanol. We focused on c118 not only because of its preferential expression in the ethanol-administered NA but also because this gene, which encodes a KH domain-containing RNA binding protein QKI-5A, has been isolated as a candidate gene for mouse neurological mutation quaking, which exhibits severe dysmyelination of the central nervous system.[27,28] QKI proteins are considered to be regulators of myelination and their absence causes dysmyelination. Hence, their up-regulation may offer protection against ethanol-induced dysmyelination. It therefore appears to be important to assess their function as well as gene regulation in the chronically ethanol-administered brain.

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