

Characterization of the Two Genes Differentially Expressed During Development in Human Fetal Astrocytes

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Astrocytes are ubiquitous in the brain and have multiple functions. It is becoming clear that they play an important role in monitoring the neuromicroenvironment, information processing, and signaling in the central nervous system (CNS) in normal conditions and that they respond to CNS injuries. During the development of the CNS, astrocytes play a key role as a substrate for neuronal migration and axonal growth.

To identify genes that could participate in astrocyte maturation, we used the differential display reverse transcription-PCR (DDRT-PCR) method. Human fetal astrocytes were cultured and total RNAs were isolated at intervals of 5 days for 50 days. Using 24 primer combinations, we identified a set of 18 candidate cDNAs deriving from the excised DDRT-PCR bands. DNA sequencing revealed 16 genes that have been described already. We found that *RTP*, *TG*, *hTM- α* , *SPARC*, *TRIP7*, and *RPL7* genes were expressed increasingly, while *HMGCR*, *RPL27a*, *NACA*, *NPM*, and *TARBP2* genes were expressed decreasingly, according to their culture stages. We also found two unidentified genes, A3 and C8, which were expressed differently in culture stages; the former was expressed decreasingly and the latter increasingly. These two genes were found in the same amount in genomic DNA from various human cells such as astrocytes, astrocytoma, trophoblasts and lymphocytes. The A3 gene was found only in human genomic DNA, but not in rat (ATr5), mouse (RAW264.7), or monkey (Vero) cells, whereas the C8 gene was found in human genomic DNA and monkey cells, but not in rat or mouse cells.

We analysed these two genes for identification. There was

>92% nucleotide sequence identity between the A3 gene (3,626 bp) and the Homo sapiens general transcription factor 3 (*GTF3*), and >96% nucleotide sequence identity between the C8 gene (2,401 bp) and the transmembrane receptor Unc5h2. These findings suggest that these two genes may participate in some functional roles within the cells.

Key Words: Astrocytes, DDRT-PCR, RACE, gene sequence.

INTRODUCTION

The central nervous system (CNS) is made up of several cell populations, mainly neurons, and microglial and macroglial cells. Astrocytes are the principal macroglial cells and the major glial cells within the CNS. Many functions have been attributed to astrocytes, including cellular support during CNS development, ion homeostasis, uptake of neurotransmitters, contribution to the CNS immunosystem and neuromodulation.¹ Astrocytes affect neuronal function by the release of neurotrophic factors, guide neuronal development, contribute to the metabolism of neurotransmitters, and regulate extracellular pH and K⁺ level.² Astrocytes are offered by glial scaffold for neuroblast migration, and play a constitutive role in the formation of the blood brain barrier.³ Astrocytic glutamate transporters that are predominantly coupled to Na⁺-dependent system mediate astrocytic glutamate uptake.⁴ In addition, astrocytes can release a variety of modulatory substances, including neurotransmitters such as adenosine triphosphate (ATP) and glutamate, growth factors such as nerve growth factor (NGF), neurotrophin-3 (NT-3) and basic fibroblast growth

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factor (bFGF), and cytokines (ICAM), to which neurons respond.⁵⁻⁹ Astrocytes also play a role in CNS injury and regeneration. After insult or trauma, astrocytes become activated and endeavor to restore the brain's delicately balanced microenvironment. Many factors have been attributed to astrocyte differentiation during CNS development. Transcription factors promote astrocyte differentiation from glioblasts, and LIF acts cooperatively with bone morphogenetic protein (BMP) 2 to induce astrocyte differentiation in the developing brain.¹⁰ Astrocytes undergo changes in morphology during both normal brain development,¹¹ and various pathological and physiological conditions.¹² Various extrinsic factors, including, among others, growth factors,¹³ neurotransmitters,¹⁴ hormones,¹⁵ transcriptional regulators,¹⁶ cyclic-AMP derivatives,¹⁷ and phorbol esters,¹⁸ have been suggested to have a profound effect in the regulation of astrocyte morphology, leading to differentiation and maturation of these cells.

Knowledge of astrocyte function has been enhanced from studies using primary cell cultures, which have many advantages, but the regulation gene for astrocyte functions remains to be elucidated. In this study, we applied differential display reverse transcription-polymerase chain reaction (DDRT-PCR) to search for the genes involved in astrocyte development in human fetal astrocytes.

MATERIALS AND METHODS

Cell culture

Human fetal astrocytes were isolated from the brain tissue of human fetuses which were aborted for therapeutic purposes between 20 and 25 weeks. The purity of astrocytes was assessed by staining for glial fibrillary acidic protein (GFAP), an astrocyte-specific protein¹⁹; the cultured cells were >95% GFAP positive.²⁰ Human lymphocytes were isolated from healthy adults. HTR-8/SV neo cells were derived from human, first trimester, extravillous trophoblasts and were immortalized by transfection with pSV3neo.²¹ Human astrocytoma U-87MG cells, Monkey Vero cells, rat ATr5 cells and mouse RAW264.7 cells were purchased from American Tissue Culture Collection

(ATCC) (Manassas, VA), and maintained according to the manufacturer's instructions. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM nonessential amino acid, 50 U/ml penicillin and 5 mg/ml streptomycin.

RNA isolation

Total cellular RNAs were isolated by Trizol reagent. Briefly, cells were washed once in phosphate buffered saline (PBS) and lysed directly in culture dishes. RNAs were extracted using 1 ml of Trizol reagent per 1×10^6 cells and the amount of RNA was determined by spectrophotometry. RNAs of human fetal astrocytes were isolated at intervals of 5 days for 50 days.

Genomic DNA isolation

Cells were washed once with PBS and lysed in the protein precipitation buffer for protein removal and genomic DNA isolation on isopropanol precipitation reaction. The amount of genomic DNA was determined by spectrophotometry after being dissolved in DNA rehydrate solution. A part of the genomic DNAs was qualitatively analyzed in gel electrophoresis.

Differential display reverse transcription-PCR

A GenHunter RNAimage kit (GenHunter Co., Nashville, TA, USA) was used for DDRT-PCR. After synthesizing the cDNA by reverse transcription using 3 types of oligo-dT primer (HT11G-AAGCTTTTTTTTTTTG, HT11C-AAGCTTTTTTTTTTTC, HT11A-AAGCTTTTTTTTTTTTA) according to the manufacturer's instruction, the 3 oligo-dT primers, and 8 types of arbitrary primer (HAP1-AAGCTTGATTGCC, HAP2-AAGC TTCGACTGT, HAP3-AAGCTTGGTCAG, HAP4-AAGCTTCTCAACG, HAP5-AAGCTTAGTAGGC, HAP6-AAGCTTGCACCAT, HAP7-AAGCTTAACGAGG, HAP8-AAGCTTTTACCGC), were combined for each PCR. PCR was performed in 40 cycles at 94°C for 30 sec, at 40°C for 2 min, and at 72°C for 30 sec; and finished at 72°C for 5 min. The amplified cDNA underwent electrophoresis on 6% dena-

turing polyacrylamide gel. After the gel was dried, it was exposed to X-ray film for 24 h to select the bands showing the locations with the specific expression of interest. The same location of each gel was cut out, for comparison with the isolated band, placed in 100 μ l of TE buffer, boiled for 15 min, and used as the template for the second amplification. After the addition of 4 μ l of the extracted cDNA, 20 μ M dNTP, 0.2 μ M arbitrary primer, 0.2 μ M oligo-dT primer and 0.4 μ l Taq DNA polymerase (5 U/ μ l; Takara Bio Inc., Shiga, Japan), the final volume was adjusted to 40 μ l. The second amplification was performed under the same PCR conditions.

cDNA synthesis and PCR

Reverse transcription of 2 μ g of RNA was performed with 1 μ g oligo-dT primer followed by the addition of a reaction mixture containing buffer, 40 U of RNase inhibitor, 20 U of MMLV reverse transcriptase, and 1 mM dNTP mix in a final volume of 25 μ l. The mixture was incubated at 42°C for 1 hr and terminated at 95°C for 5 min. Amplifications of A3 gene, C8 gene, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from cDNA and genomic DNA were performed using an automatic thermocycler (Hybaid, Teddington, UK) in a reaction mixture containing PCR buffer, 0.2 mM dNTP, 1 U of taq polymerase (Takara Bio Inc., Shiga, Japan), 0.25 μ M primer, cDNA or genomic DNA and H₂O. The A3 and C8 gene PCR products were amplified for 30 cycles at 94°C for 30 sec, 48°C for 30 sec, and 72°C for 30 sec, followed by 7 min extension at 72°C. The GAPDH PCR product was amplified for 25 cycles at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 60 sec, followed by 7 min extension at 72°C. All of the PCR products were viewed under ultraviolet light after 1.5% agarose gel electrophoresis and staining in ethidium bromide. The PCR primer sets were 5'-TAATGTATGAGAGGACCCTC-3' (forward), 5'-CAATACTAATAAAACATGCA-3' (reverse) for A3; 5'-TAGTAAGGCTCAAAGAAGAA-3' (forward), 5'-TTAAAAAGGACTTATCCAAA-3' (reverse) for C8; and 5'-ACCACAGTCCATGCCATCAC-3' (forward), 5'-TCCACCACCCTGTTGCTGTA-3' (reverse) for GAPDH. The semi-quantification results were analyzed using the Quantity One Software

(Bio-RAD, Hercules, CA, USA).

Rapid amplification of cDNA ends (RACE)

cDNA was synthesized by reverse transcription using A3 and C8 gene specific reverse primers and RNA. First strand cDNAs were purified by RNA degradation for RNase H. 5'-RACE cDNAs were supplemented by anchor sequence in 1st strand cDNAs for Terminal deoxynucleotidyl transferase (TdT) to nucleotide homopolymer (dATP). The 3'-RACE cDNAs were synthesized using a traditional reverse transcription procedure, but with a special oligo-dT primer (5'-GACTCGAGTCGACATCGACT(T)₁₇-3'). Amplification of 5'- and 3'-RACE cDNAs was performed using an automatic thermocycler in a reaction mixture containing PCR buffer, dNTP, taq polymerase (dT)₁₇-adaptor primer (5'-GACTCGAGTCGACATCG(T)₁₇-3'), adaptor primer (5'-GACTCGAGTCGACATCG-3'), gene specific primer, RACE cDNA and H₂O. The amplification was performed with a first cycle at 94°C for 5 min, 50°C for 5 min, and 72°C for 40 min, 30 subsequent cycles at 94°C for 40 sec, 50°C for 1 min, and 72°C for 3 min, and finally a 10-min extension for 72°C. All of the amplification products were viewed under ultraviolet light after 1.5% agarose gel electrophoresis and staining in ethidium bromide. The amplification products were purified using a Gel Extraction kit (Hybaid, Teddington, UK). The isolated fragments were amplified using adaptor primers and gene specific primers for nested PCR. The nested PCR product were amplified for 30 cycles at 94°C for 40 sec, 48°C for 1 min, and 72°C for 3 min, followed by a 7 min extension for 72°C.

Cloning and analysis of nucleotide sequence analysis

The DDRT-PCR amplification product and the RACE product amplified for nested PCR were placed in a pGEM-T vector (Promega, Madison, WI, USA) and transformed into the prepared *E. coli* JM109 strain, using the CaCl₂ method. After selecting the transformants showing antibiotic resistance, the insertion of the PCR products was confirmed from the digestion pattern of the restriction enzymes. The nucleotide sequence of

each clone was determined using an Auto-sequencer (LONG READIR 4200, LI-COR, Lincoln, NE, USA). The analysis of the confirmed nucleotide sequences was performed by a comparison with those nucleotide sequences registered in GenBank, using the BLAST Network Service provided by the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA).

RESULTS

Comparison of mRNA Differential Display condition in primary cultured human fetal astrocytes by differential display reverse transcription-PCR

Human fetal astrocytes were isolated from the brain tissue of human fetuses between 20 and 25 weeks to identify any genes specially expressed, such as decreasingly or increasingly, during cultures. We compared the mRNA differential display pattern of 10 sets of total RNAs isolated at

intervals of 5 days for 50 days in the primary culture stage. We identified a set of 18 candidate cDNAs that were differentially expressed over their culture stages. DNA sequencing revealed 16 genes that have been described already. We found that tunicamycin-responsive protein (*RTP*), transglutaminase (*TG*), alpha-tropomyosin (*hTM- α*), secreted protein acidic and rich in cysteine (*SPARC*), a novel human gene mapping to chromosome 1, thyroid receptor interactor (*TRIP7*), and ribosomal protein L7 (*RPL7*) genes were expressed increasingly. We also found that 3-hydroxy-3-methylglutaryl-Coenzyme A (*HMG-CoA*) reductase (*HMGCR*), ribosomal protein L27a (*RPL27a*), nascent polypeptide associated complex (*NACA*), nucleophosmin phosphoprotein (*NPM*), and transactivation responsive (*TAR*) (*HIV*) RNA binding protein 2 (*TARBP2*) genes were expressed decreasingly according to their culture stages. Furthermore, we also found two unidentified genes (*A3* and *C8*) which were expressed differently in variable culture stages (Table 1).

Table 1. Identity (%) and Expression Pattern of DDRT-PCR Product Sequences in Human Fetal Astrocytes During Development Stages

Putative Identification	Expression Pattern	Putative Identification	Expression Pattern
100% homology to 3-hydroxy-3-methylglutaryl-Coenzyme A (<i>HMG-CoA</i>) reductase (<i>HMGCR</i>)	Decrease	100% homology to mRNA for tunicamycin-responsive protein (<i>RTP</i>)	Increase
99% homology to K1AA0872 mRNA	Decrease	100% homology to transglutaminase (<i>TG</i>)	Increase
99% homology to ribosomal protein L27a (<i>RPL27a</i>)	Decrease	100% homology to alpha-tropomyosin (<i>hTM-α</i>)	Increase
100% homology to TCBA00781 mRNA	Decrease	98% homology to chromosome 16 BAC clone	Increase
99% homology to nascent polypeptide associated complex (<i>NACA</i>)	Decrease	99% homology to secreted Protein Acidic and Rich in Cysteine (<i>SPARC</i>)	Increase
98% homology to nucleophosmin phosphoprotein (<i>NPM</i>)	Decrease	99% homology to novel human gene mapping to chromosome 1	Increase
100% homology to <i>TAR</i> (<i>HIV</i>) RNA binding protein 2 (<i>TARBP2</i>)	Decrease	100% homology to BAC11-1038A11	Increase
Unknown (<i>A3</i>)	Decrease	100% homology to thyroid receptor interactor (<i>TRIP7</i>)	Increase
Unknown (<i>C8</i>)	Increase	100% homology to ribosomal protein L7 (<i>RPL7</i>)	Increase

A3 and C8 mRNA expression in primary cultured human fetal astrocytes

A3 was expressed decreasingly and C8 increasingly in accordance with their culture stages (Fig. 1). RT-PCR of 9 sets of RNAs isolated from human fetal astrocytes according to culture stages was performed with A3 and C8 gene specific primers designed from the DDRT-PCR product sequence. The RT-PCR results were the same of those of DDRT-PCR. The A3 gene expression gradually decreased after a high level in the early days. A3 was not expressed after 35 days in culture. Meanwhile, the C8 gene was expressed increasingly until 20 days (Fig. 2).

Analysis of A3 and C8 genes in various human cells

We used human fetal astrocytes, U-87MG, human lymphocytes, and HTR-8/SV neo cells for analysis of A3 and C8 gene expression in various human cells.

A3 and C8 fragments were amplified from genomic DNA isolated from astrocytes, astrocytoma, lymphocytes, and trophoblasts with gene

specific primers. We detected A3 and C8 genes in DNA from all of these cells (Fig. 3). They were also detected in the total RNAs of these cells; however, the expressions were different (Fig. 4).

Expression of A3 and C8 in various species

We used Vero (monkey), Atr5 (rat), and RAW 264.7 (mouse) cells for detecting A3 and C8 gene expression in differential species.

Genomic DNA was isolated from each cell and amplified with A3 and C8 gene specific primers. Also genomic DNA of human fetal astrocytes was amplified equally for comparison. In the results, the A3 gene was found only in genomic DNA of human cells while the C8 gene was found in genomic DNA of humans and monkeys, but not in rat and mouse cells (Fig. 5).

Full sequence of A3 and C8 cDNA

We performed RACE to identify the full sequences of A3 and C8 genes. A3 or C8 cDNA ends were amplified with gene specific primer and adaptor primer in 5'-cDNA ends and 3'-cDNA ends that synthesized the total RNAs of human

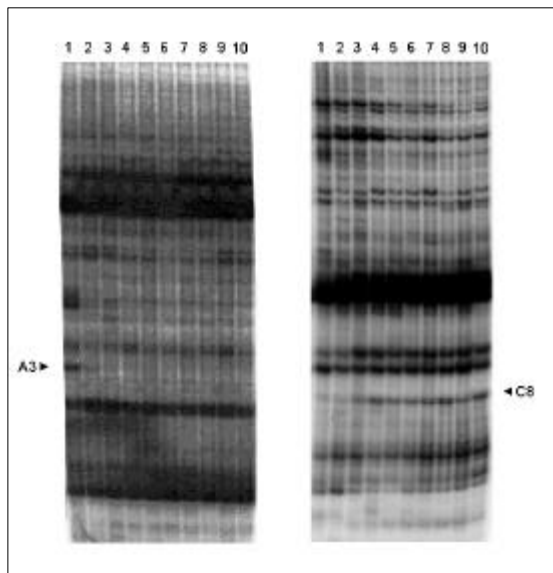


Fig. 1. Representatives of differentially displayed RT-PCR. RNAs isolated from human fetal astrocytes at intervals of 5 days for 50 days (1 to 10, culture stages). Differentially expressed genes are indicated as arrow heads. The primers were HT11A and AP3 for A3, and HT11C and AP8 for C8.

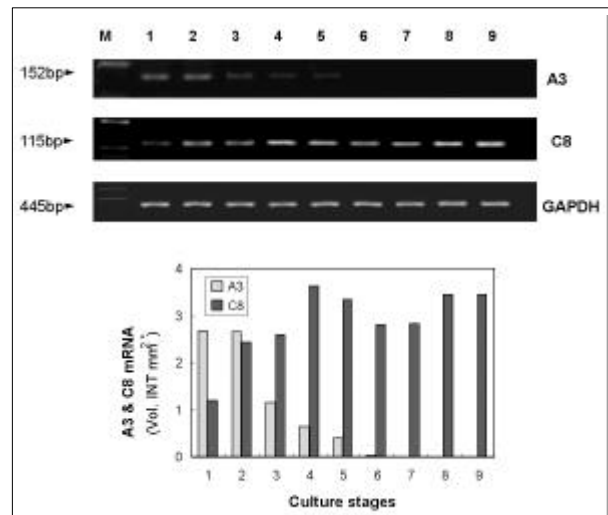


Fig. 2. A3 and C8 mRNA expression in primary cultured human fetal astrocytes. RT-PCR products amplified with cDNA synthesized from total RNAs in human fetal astrocytes using sequence specific primers (A3, C8). RNAs isolated from human fetal astrocytes at intervals of 5 day for 45 days (1 to 9, culture stages). M, 100bp DNA ladder. The relative mRNA expression levels were analysed using a densitometer. * volume intensity count per mm²

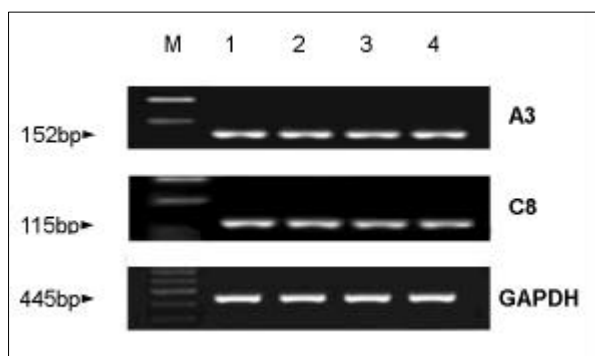


Fig. 3. A3 and C8 genomic DNA expression in various human cells. PCR products amplified from genomic DNAs using sequence specific primers (A3, C8). Genomic DNAs isolated from human fetal astrocyte (1), U-87MG (human astrocytoma, 2), human lymphocyte (3), and HTR-8/SV neo (human trophoblast, 4). M, 100bp DNA ladder.

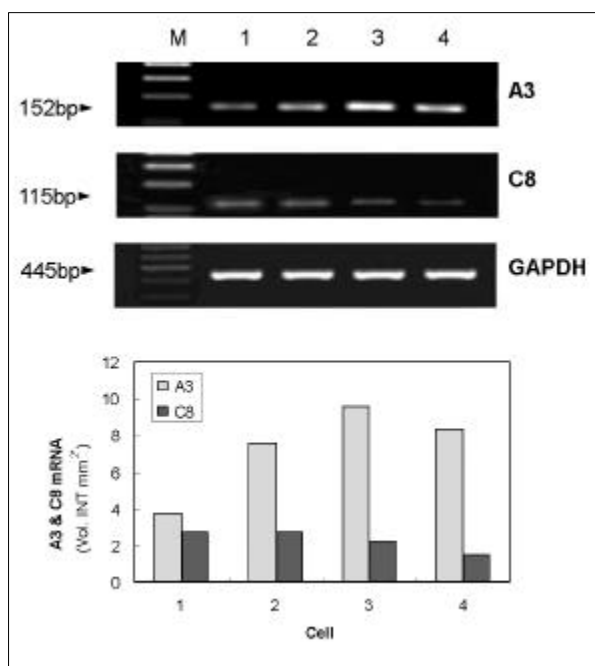


Fig. 4. A3 and C8 mRNA expression in various human cells. RT-PCR products amplified with cDNA synthesized from total RNAs using sequence specific primers (A3, C8). RNAs isolated from human fetal astrocyte (1), U-87MG (human astrocytoma, 2), human lymphocyte (3), and HTR-8/SV neo (human trophoblast, 4). M, 100bp DNA ladder. The relative mRNA expression levels were analysed using a densitometer.

fetal astrocytes. Amplification products were placed in pGEM-T vectors and the nucleotide sequence of each clone was determined. Each clone with a confirmed nucleotide sequence was compared for

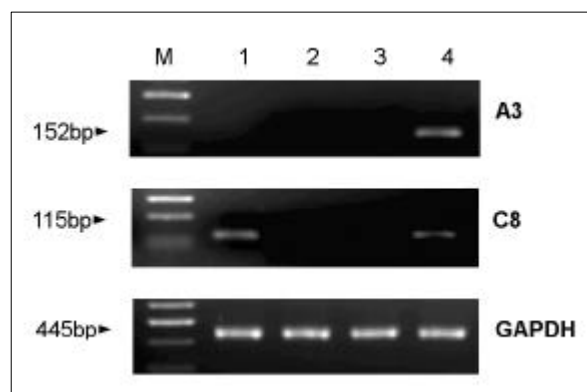


Fig. 5. A3 and C8 genomic DNA expression in monkey, rat, mouse and human. PCR products amplified from genomic DNAs using sequence specific primers (A3, C8). Genomic DNAs isolated from Vero (monkey, 1), Atr5 (Rattus norvegicus, 2), RAW264.7 (Mus musculus, 3), and human fetal astrocyte (4). M, 100bp DNA ladder

homology with the nucleotide sequences in the NCBI GenBank. The nucleotide sequence of the fragment A3 (3,451 bp) was found to be >92% identical to Homo sapiens general transcription factor 3 (*GTF3*, GenBank accession no. AF151354), and that of the fragment C8 (2,420 bp) was found to be >96% identical to 3'-end of transmembrane receptor *Unc5h2* (*Unc5h2*, GenBank accession no. AY126437) (Fig. 6).

DISCUSSION

Astrocytes are the most prominent glial cells in the CNS and are involved in immunological and inflammatory events occurring within the CNS. Astrocytes play an important role in monitoring the neuromicroenvironment in CNS and in information processing or signaling in the nervous system in normal conditions and they respond to CNS injuries in a gradual and varied way.¹

This research was focused on identifying genes that could participate in astrocyte maturation. We identified a set of 18 candidate cDNAs deriving from excised DDRT-PCR bands. DNA sequencing revealed 16 genes that have been described already and 2 unidentified genes. We found that *RTP*, *TG*, *hTM-α*, *SPARC*, a novel human gene mapping to chromosome 1, *TRIP7*, and *RPL7* genes were expressed increasingly according to

A3	:	2998	ATGTCGCGCATGGTCATCATTAACACAGCTCCAACCCCTTGCAGAAATCTGCAATGATGCCAAGGTGCCAGCCAAAGACAGC-----AGCATGCCAAGCGCAAGAGAAGCGGGTC	3107
GTF3	:	3000	*****-*****CAAAGACAGC*****	3118
A3	:	3108	TCGGAAGGAAATCCGTCCTCTTCTCCTCGTCTTCTCTTCTCGTCCCTCTAACCCGATTTCAGTGGCATCGGCCAACAGATCTCACTCGTCAATGGCCAATGTACATGGTGAC	3227
GTF3	:	3119	*****C*****	3238
A3	:	3228	TATGCGCGCTGAACGTGCAGCTCCGGACCTCTTAATTACTAGACCT--CAGTACTGAATCAGGACCTCACTCAGAAAGACTAAAGGAATGTAATTTATGTACAAATGTATATTGC	3345
GTF3	:	3239	*****C*****CA*****-*****	3357
A3	:	3346	-GATATGTATCGATGCCTTTTAGTTTTTCCAATGATTTTACACTATATTCCTGCCACCAAGGCCTTTTAAATAAGTTAATGTATGAGAGGACCTCTGCTGGCTGTGAAAGTCTCAGT	3464
GTF3	:	3358	A*****AAAAAAAAAAAAAAAAAAAA	3446
A3	:	3465	CCCCAGGATAGTGAAGGATAAAAGTAATCTCGTTGTGTTACCATTTTTCAGGATCTGCTATACCCATACATTTACTGGGTGCTTTATGCATGTTTACACTATATTCCTGCCACC	3584
A3	:	3585	AAGGCCTTTTAAATAAGTAAAAAAAAAAAAAAAA	3626
A				
C8	:	1919	TAGAAAAATCCATGTACTCTGTGTGTAGAGGGCCAGAGTTCCTTCTCCACCCCG-----TGGCCTGAGATCTCTGTGCAGGAACCAAGATGGGGCTGAAGCCTCTGGAGGCA	2026
Unc5h2	:	3026	*****CCTCTCTCTCT*****	3145
C8	:	2027	GTGTGTGGGGCGGGCAGGAGGCCCTCCCTCCA-----ACCTCAGCCGGCAACTCTGGGTTCATGGGTTT-AGTTCCGTTC-TCGTTTCTCTCCGTTATGA-TT	2136
Unc5h2	:	3146	*****CCCCC*****-*****A*****T*****-*****T**	3263
C8	:	2136	TCCTC-TTCTCCCTAAGCCCTCTCTGCTTCCAGCCCTTTTCTCTTTGAAGAGTCAAGTACAA-TTCAGACAAAC-TGCTTCTCTG-TCCAAAAGCAAAAGGCAAAAGGAAGAA	2253
Unc5h2	:	3263	****T**T*****-*****-*****T*****-*****T*A*A*****T*****T*T**T*****CA****-*****-*****A**	3379
C8	:	2254	AGAAAGCTTCAGACCGCTAGTAAGGCTCAAAGAAGAAGAAAAACACCAAAACCACAAGGGAAAAGAAAGCCGAGTTTCTTAGGAAACGCAACAGATTATTATCCAGATTATTGGATA	2373
Unc5h2	:	3380	*****A****T*T*****T*-*****A*A****C*CT*C*CC*+C*CCC*C*GCCCGCAACTC*GGG*TCG*GG*TTTTA*T+C*CG*T*T*TTCC*CG*TAITG	3498
C8	:	2374	AGTCCTTTTTAAGAAAAAAAAAAAAAAAA	2401
Unc5h2	:	3500	*T*T**CC**TCTCCCT**GCCCTTCT	3526c
B				

Fig. 6. Alignment of the predicted nucleotide sequences. (A) Comparison of parts of the nucleotide sequences of A3 cDNA in the primarily cultured human fetal astrocytes by rapid amplification of cDNA ends with GTF3. A3 (3,451 bp) shows >92% homology with Homo sapiens general transcription factor 3 (GTF3, GenBank accession no. AF151354). (B) Comparison of parts of the nucleotide sequences of C8 cDNA in the primarily cultured human fetal astrocytes by rapid amplification of cDNA ends with Unc5h2. C8 (2,420 bp) shows >96% homology with transmembrane receptor Unc5h2 (Unc5h2, GenBank accession no. AY126437). Identical nucleotides are asterisked (*) and gaps introduced to maximize homology are indicated by dashes (-).

their culture stages. The expression of RTP mRNA in syncytiotrophoblasts and its up-regulation during forskolin-induced BeWo cell differentiation suggest a physiological role in trophoblast differentiation.²² Similarly, the expression of RTP in human fetal astrocytes suggested that they might have a physiologic role in astrocytes differentiation. Tissue transglutaminase (TG), a multifunctional protein, is likely to play a role in numerous processes in the nervous system, and is a highly regulated and inducible enzyme especially in the development of the nervous system. Recent findings have provided evidence that dysregulation of tissue TG might contribute to the pathology of several neurodegenerative conditions including Alzheimer's disease and Huntington's disease. In

both of these diseases tissue TG and TG activity are elevated compared to age-matched controls.²³ Tropomyosins, a multigene family of coiled-coil, actin filament-binding proteins, regulate actomyosin function and stabilize the actin.^{24,25} The role of tropomyosins is well established in skeletal muscle contraction, but its function in nonmuscle cells is unknown.²⁶ SPARC, a matricellular protein that affects cellular adhesion and proliferation, is produced in remodeling tissue and in pathologies involving fibrosis and angiogenesis.²⁷ The expression of *SPARC* in astrocytes suggested that the SPARC dynamic acts in premature nervous system. RPL7 takes part in transcription and translation.²⁸ Increasing RPL7 expression coincides with gene participation in cell growth and prolifera-

tion, and in tumor formation.

We found that *HMGCR*, *RPL27a*, *NACA*, *NPM*, and *TARBP2* genes were expressed decreasingly according to their culture stages. *HMGCR* catalyzes the rate-limiting reaction of the cholesterol synthesis pathway. As development occurs, this microsomal enzyme is expressed in all cell types during membrane synthesis, such as during cell growth and division.²⁹ In the brain, several studies have demonstrated enhancement of *HMGCR* activity and expression of the *HMGCR* gene in oligodendrocytes during myelination.³⁰ Messenger RNA levels of *HMGCR* in the brain decreased with age, and those levels at -5 (5 days before birth) and 5 days after birth were significantly higher than the control level of adult mice. The period from -5 to 5 days might correspond to stages of active biogenesis of the membranes of brain cells. The mRNA level of *HMGCR* in the liver was also high at -5 days; a finding that correlated with cell proliferation.³¹ Similarly, the expression of *HMGCR* gene in human fetal astrocytes decreased with advancing development stages. Therefore, *HMGCR* correlated with astrocyte proliferation. *RPL27a* takes part in transcription and translation³² and *TARBP2* influences gene expression that accounts for binding with RNA.³³ *NACA* is a dynamic component of the ribosomal exit tunnel, providing a shield for nascent polypeptides, and is a negative regulator of translocation into the endoplasmic reticulum and a positive regulator of translocation into the mitochondria.³⁴ *NPM* is a major nuclear protein which is 20 times more abundant in tumors or in proliferating cells than in normal resting cells. Recently, it was found that the *NPM* gene is located at the breakpoints of the t (2:5), t (3:5) and t (5:17) chromosome translocation. *NPM* biosynthesis is related to cell proliferation and mitogenesis. *NPM* mRNA is 50-fold higher in Novikoff hepatoma and 5-fold higher in hypertrophic rat liver than in normal rat liver.³⁵ Feuerstein, et al.³⁶ reported that when B cells, T cells and Swiss 3T3 cells were stimulated with various mitotic agents, *NPM* synthesis increased. On the other hand, down regulation of *NPM* was observed in Jurkat T-lymphoblasts during apoptosis.³⁷ These studies indicate that *NPM* expression is associated with cell growth.

The *TG*, tropomyosin, *SPARC*, *TRIP7*, and

HMGCR genes play a constitutive role in the formation of the brain, while the *RPL7*, *RPL27a*, *NACA*, and *NPM* genes regulate the gene expression. Therefore, we suggested that the unidentified genes (A3 and C8) might play a role in brain formation or the regulation of gene expression. In this study, we found that A3 was expressed decreasingly and C8 increasingly in accordance with culture stages. These results suggest that the A3 gene could be involved at the early stage of astrocyte differentiation, and that the C8 gene could be related to astrocyte development and play an important role with passing time.

The A3 and C8 genes underwent RACE to identify the full sequence, and their nucleotide sequences were analyzed by searching for homologies against the GenBank data bases. The nucleotide sequence of the fragment A3 was found to be > 92% identical to Homo sapiens general transcription factor 3 (*GTF3*, GenBank accession no. AF151354), a multidomain nuclear protein related to initiator element-binding transcription factor TF II-I. The genes for both proteins are deleted in persons with Williams-Beuren syndrome who often manifest muscle weakness. General transcription factor 3 expression is neither muscle- nor fiber-type specific. Its levels are highest during a period of fetal development that coincides with the emergence of specific fiber types and transiently increase in regenerating muscles damaged by bupivacaine.³⁸

The nucleotide sequence of the fragment C8 was found to be > 96% identical to 3'-end of transmembrane receptor *Unc5h2* (*Unc5h2*, GenBank accession no. AY126437), a novel inhibitor of $G_{i\alpha 2}$ that thereby increases intracellular cAMP levels. *Unc5h2* is widely expressed in many tissues in addition to the brain, including leukocytes and the lung where cell migration is important. The expression of *Unc5h2* in the brain and immune system suggests that this novel inhibitor of G protein signaling may have broad significance for axonal guidance and chemotaxis.³⁹ The most widely expressed *Unc5* family member is *Unc5h2* and its mRNA is observed during early blood vessel formation, in the semicircular canal and in a dorsal to ventral gradient in the retina. *Unc5h1* expression is restricted to the CNS, whereas, sites of *Unc5h4* expression are in the

developing limb and mammary gland.⁴⁰

We amplified A3 and C8 genes after genomic DNA and total RNA were extracted from human fetal astrocytes, human astrocytoma (U-87MG), human lymphocytes, and human trophoblasts (HTR-8/SV neo) for analysis of A3 and C8 expression patterns in various human cells. The amplification results indicated that Genomic DNAs have equal expression and that amplified total RNAs have different expression. Therefore, we suggested that the A3 and C8 genes play specific roles. The A3 gene was expressed more lymphocytes than other cells and A3 gene nucleotide sequences were almost identical to a part of general transcription factor 3 whose expression levels, as described above, are highest during a period of fetal development that coincides with the emergence of specific fiber types. Therefore, the A3 gene is suggested to play a role in the regulation of astrocyte morphology. The C8 gene was expressed more astrocytes and astrocytoma than other cells and C8 gene nucleotide sequences were almost identical to 3'-end of transmembrane receptor *Unc5h2*, a novel inhibitor of G protein signaling that may have significance for axonal guidance and chemotaxis and whose mRNA is observed during early blood vessel formation. These results suggest that the C8 gene may play a role in astrocytes migration. Astrocytes play a constitutive role in the formation of the blood-brain barrier.⁴¹ We suggested that the C8 gene participates in astrocyte's role in the formation of the blood-brain barrier.

We amplified A3 and C8 genes after genomic DNA were extracted from Vero (monkey), Atr5 (rat), and Raw264.7 (mouse) cells to investigate variations in A3 and C8 expression patterns in different species. In these results, the A3 gene was amplified only in genomic DNA of astrocytes (human), indicating that A3 has species specificity. The C8 gene was amplified in genomic DNA of astrocytes (human) and Vero (monkey), but not in Atr5 (rat) and Raw264.7 (mouse). The overall sequence homologies to *Unc5h1*, *Unc5h2* and *Unc5h3* were 44, 48.7, and 47.6%, respectively, and *Unc5h2* had a high sequence identity between rat and mouse and a low sequence identity with human.⁴⁰ The results indicated that C8 amplified from genomic DNAs corresponded to a low sequence

identity of *Unc5h2*.

We investigated the identification and characterization of specially expressed genes in human fetal astrocytes during development. We applied a sensitive technique-mRNA differential display analysis-to evaluate changes in gene expression during induction of astrocyte culture stages. We identified a set of 16 genes that have already been described, as well as 2 unidentified genes, A3 and C8. Further investigations are required to analysis the gene expression pattern in astrocytes, neurons, or oligodendrocytes, and such experimental results may provide clues to the pathology and physiology of degeneration of several neurodegenerative conditions including Alzheimer's disease and Huntington's disease.

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