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Cloning of *LASS1* Gene and Primary Study on The Association of Its Expression With Neuron Aging in Rat Cerebral Cortex

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Abstract *LAG1* was identified as a gene that is differentially expressed during the yeast replicative life span and was shown to play a role in determining yeast longevity. The cDNA of rat *LASS1*, the mammalian homolog of yeast *LAG1*, was cloned from rat cerebral cortex and sequenced, which is different to the predicted sequence in the GenBank. Sequence analysis revealed that this cDNA clone contains an open reading frame of 1 053 bp. The deduced amino acid sequence has 350 residues and shares a predicted Lag1p motif and a TLC domain conserved in Lag1 proteins. Total RNAs were isolated from rat cerebral cortices at varying ages: newborn, one month, six months, twelve months, and twenty-four months. Semi-quantitative RT-PCR and Northern blot analysis were performed to analyze the *LASS1* expression level in rat cerebral cortex tissues at varying ages. Senescence-associated β-galactosidase (SA-β-gal) activity was firstly used as a biomarker for assessing senescence in rat neurons. The results showed that *LASS1* expression was upregulated from newborn to adult rats (1～6 month) and declined in aged cortex. SA-β-gal staining positive neurons significantly increased in the aged cerebral cortex. The age-related expression alternation of *LASS1* in rat cerebral cortex provides an important clue in exploring the role of *LASS1* in mammalian neuron aging.

Key words *LASS1* cloning, rat, gene expression, neuron senescence

Longevity-assurance gene 1 (*LAG1*) was the first of several longevity genes identified and cloned from the baker's yeast *Saccharomyces cerevisiae* [1]. Homologues of *LAG1* have been identified in several species, and each species appears to possess more than one homologue. *LAC1* is the close homologue of *LAG1* in yeast[2~6]. The mammalian homologues have been renamed as *LASS1*, *LASS2* and *LASS4*～*6*[5]. In adult human tissues, *LASS1* was expressed only in brain, skeletal muscle, and testis[3]. *LAG1* attracted attention because of its expression sharply declines later in yeast life span[1,7]. The overexpression of *LAG1* had a bimodal effect on yeast, with moderate expression resulting in increased longevity and with higher expression curtailing life span[8]. However, deletion of *LAG1* was found to extend yeast life span[1]. Deletion of its close homologue *LAC1* does not affect yeast life span, but the double deletion mutant *lag1Δ lac1Δ* is inviable[3].

There is little overall sequence similarity between Lag1 homologue proteins from various species[2~6]. However, all these proteins possess a stretch of 52 amino acids of high sequence similarity, which has been dubbed the Lag1p motif[3]. In recent years, it was shown that Lag1p and Lac1p are located in the endoplasmic reticulum (ER) and that their deletion reduces the rate of transport of glycosylphosphatidylinositol (GPI)-anchored proteins from the ER to the Golgi[9]. As the transport of GPI proteins is singularly dependent on sphingolipids[10], this observation led to the realization that Lag1p and Lac1p may be an essential component of ceramide

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synthase[11,12], or subunits of the enzyme[13]. Studies also revealed that lag1p and its homologs possess a characteristic substrate preference for a particular fatty acyl-CoA in the process of ceramide synthesis [5,14]. Mutant *lag1Δ lac1Δ* cells have reduced sphingolipid levels due to a loss of the Fumonisin B1-sensitive and C26-CoA dependent ceramide synthase reaction [11,12]. The LASS1 protein selectively regulates the synthesis of C18:0-containing sphingolipids in cultured mammalian cells [15], while microsomes made from *LASS1*-overexpressing *lag1Δ lac1Δ* yeast cells mainly exhibited C26:0-CoA-dependent ceramide synthase activity[16]. Ceramide functions as a second messenger in a variety of cellular events including apoptosis and differentiation [17 ~19]. Changes of the yeast life span affected by overexpression of *LAG1* might be associated with subtle changes in ceramide/ sphingolipid metabolism [8,12]. But the mechanism by which *LAG1* determines life span and the relationship between longevity and ceramide biosynthesis are still unclear at present.

Ceramides have been implicated in various neuronal events, such as the pathological death of neurons that occurs in ischemic stroke [20] and Parkinson's disease [21]. Previous data shows that in brain C18:0-ceramide is the major ceramide [22,23]. *LASS1* regulates C18:0-ceramide synthesis, and is expressed in brain abundantly and specifically[5]. We wonder whether *LASS1* may participate in the process of mammalian neuron aging.

The sequence of rat *LASS1* mRNA has not been cloned yet (only predicted sequence in GenBank). In this study, we cloned the *LASS1* cDNA (GenBank accession number: DQ479969) derived from mRNA of rat cerebral cortex. Semi-quantitative RT-PCR and Northern blot analysis were performed to analyze the *LASS1* transcription levels in rat cerebral cortex tissue at varying ages. The senescence-associated β-galactosidase activity was histochemically examined for the first time as a cell senescence marker in rat brain tissues. Our data suggest that rat neuron aging might be associated with the alternation of *LASS1* expression.

1 Materials and methods

1.1 Animals

All animal procedures were approved by The Animal Care and Use Committee of Shantou University Medical College. Male Sprague-Dawley rats were classified into five age-groups, they are newborn rats (postnatal age, 1 day), 1 month, 6 months, 12 months, and 24 months. Each group contains four rats. Rats were sacrificed by decollation under pentobarbital anesthesia and the cerebral cortex tissues were immediately removed for experiments as described below.

1.2 X-gal staining for β-galactosidase activity in brain tissue

For SA-β-gal staining[24], brain tissues of rats with ages of newborn, 1 month, 12 months, and 24 months were rapidly frozen in liquid nitrogen. Sections (6 μm) were cut, mounted onto glass slides, fixed in 4% phosphate-buffered paraformaldehyde for 1 min at room temperature, washed in PBS, immersed overnight in SA-β-Gal staining solution (1 g/L X-gal, 40 mmol/L citric acid/sodium phosphate, pH 6.0, 5 mmol/L potassium ferricyanide, 5 mmol/L potassium ferrocyanide, 150 mmol/L NaCl, 2 mmol/L $MgCl_2$), counterstained with nuclear fast red, and viewed under a microscope and photographed.

1.3 Isolation of total RNA

Total RNA was isolated from each cerebral cortex tissue using the Qiagen's RNeasy Mini Kit (Qiagen, GmbH, Germany) and was stored at −80℃ before use. RNA concentrations were determined from the absorbances measured at 260 nm and 280 nm.

1.4 Molecular cloning of *LASS1* and sequence analysis

First-Strand cDNA was synthesized from 1 μg of total RNA (from a 6-month rat cerebral cortex as described above) by the LD-PCR amplification method using the Smart cDNA library construction kit (Clontech, USA). Gene-specific primers for *LASS1* were designed from the predicted sequence (GenBank accession number: XM_224734). The sequences of sense and anti-sense primers were 5′ ACTCGGTGG-TCATGCTGGTA 3′ and 5′ GACGTCATGCAGG-AAGAACA 3′ respectively. Paired with the SMART anchor sequence at the 3′ or 5′ end, PCR techniques were performed from the First-Strand cDNA. The PCR products were purified from agarose gels using a QIAquick gel extraction kit (QIAGEN GmbH, Germany), cloned into the TA vector pGEM-T (Promega, USA), and sequenced on an ABI 377 DNA

sequencer (Perkin-Elmer Applied Biosystems) by the GeneCore Company (Shanghai, China).

The sequence of rat *LASS1* cDNA was compared with the Nucleotide data bases using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST/). The conserved domain of the deduced peptide sequence was analyzed using RPS-BLAST and TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/) algorithms.

1.5 Semi-quantitative RT-PCR analysis

To insure that no false positive PCR fragments would be generated from pseudogenes in contaminating genomic DNA, gene-specific primers for *LASS1* and 18 S rRNA (as internal control) were designed to span intron regions. The GenBank accession numbers and sequences of primers are shown in Table 1. All primers were synthesized by Invitrogen Biotechnology Co. Ltd. (China). First-strand cDNA was synthesized from 2 μg of total RNA (as described above from each rat cerebral cortex) using SuperScript Ⅲ First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, California, USA). PCR amplifications were performed in duplicates on a PTC-150 thermalcycler (MJ Research Inc, MA, USA). Using 1 × Taq Platinum PCR MasterMix (Tianwei Corp, Beijing), the 25 μl reactions contained 400 nmol/L gene specific forward and reverse primers of *LASS1* or 18 S rRNA, and 1 μl of cDNA as a template. The PCR program initially started with a 94℃ denaturation for 5 min, followed by 15 to 32 cycles of 94℃/1 min, 60℃/1 min, 72℃/1 min. Linear amplification range for each gene was tested. The PCR samples (10 μl each) were electrophoresed on 2.5% agarose gels, stained with ethidium bromide, and photographed on top of a 280 nm UV light box. The gel images were digitally captured with a CCD camera and analyzed using FluorChem 8900 (Alpha Innotech, San Leandro, CA, USA) and calculated using the integrated density value (IDV). RT-PCR values are presented as a ratio of the *lass1* signal divided by the 18S rRNA signal in the selected linear amplification cycle.

Table 1 Primer sequences of the studied genes

Gene	Accession number (GenBank)	Primer sequences	PCR fragment
LASS1	XM_224734	Forward: 5′ ACTCGGTGGTCATGCTGGTA 3′	110 bp
		Reverse: 5′ GACGTCATGCAGGAAGAACA 3′	
18S rRNA	M11188	Forward: 5′ ATTCCGATAACGAACGAGAC 3′	133 bp
		Reverse: 5′ GGCATCACAGACCTGTTATTG 3′	

1.6 Northern blot analysis

Total RNA (30 μg) prepared above (newborn, 1 month, 6 month, 12 month, 24 month age groups) was electrophoresed through a 1.2% denaturing formaldehyde agarose gel and transferred to an Immobilon-Ny$^+$ nylon membrane (Millipore, USA). RNA Ladder (Toyobo, Osaka, Japan) was used as size standards. The RNA was fixed by ultraviolet cross-linking. To generate biotinylated hybridization probes, template DNAs of *LASS1* and 18 S rRNA were generated by PCR with above described primers. DNA probes were biotinylated using a NEBlot Phototope labeling kit (New England Biolabs, USA) as recommended by the manufacturer. The membrane was prehybridized for 1～2 h and hybridized with the denatured biotinylated *LASS1* probe by standard procedures according to the NEBlot Phototope kit instruction manual. Chemiluminescent detection using the Phototope-Star Detection Kit (New England Biolabs, USA) was performed by exposing the membrane to X-ray film. The membrane was stripped and re-hybridized with the denatured biotinylated 18 S rRNA probe.

2 Results

2.1 Age-related SA-β-gal expression in rat cerebral cortex

SA-β-Gal-stained sections were examined for the staining frequency and identity of positive cells (Figure 1a). Blue positive stained neuron cells in rat cerebral cortex were counted in high-power fields (400 ×). Figure 1b indicated positive stained neuron cells per high-power field of each group (5 high-power fields of each section were counted, n = 4 for each group). Positive staining could not be found in newborn rat

cerebral cortex, and only a few lightly stained cells were found in young rats (1 month). The staining significantly increased with age in frequency and intensity (12 month and 24 month).

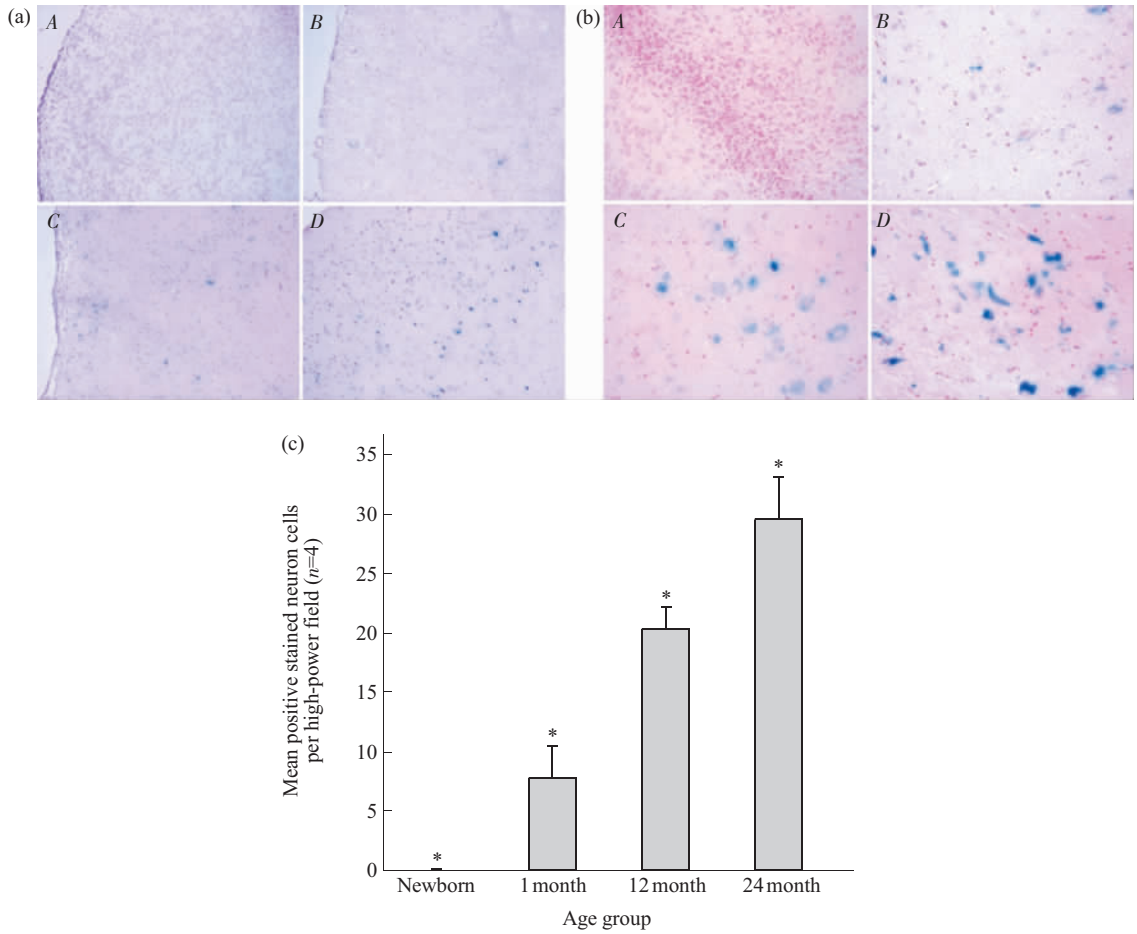

Fig. 1 SA-β-Gal in rat cerebral cortex

Rat brain sections were stained for SA-β-Gal, counterstained with nuclear fast red, and photographed at 20× (a), and 40× (b) magnification. Photographs showed stained rat cerebral cortex at age of : *A*, newborn; *B*, 1 month; *C*, 12 months; *D*, 24 months. Positive cells were blue stained, 5 high-power fields of each section were counted, $n = 4$ for each group; positive stained neuron cells in rat cerebral cortex were counted per high-power fields (c); $*P < 0.05$ (Student-Newman-Keuls test).

2.2 Sequence analysis

Similar to the mouse *LASS1* mRNA (GenBank accession number: NM_138647), the cDNA sequence contains two nonoverlapping open reading frames (ORF) of a conserved LASS1-GDF1 bicstronic strcture[4]. The ORF of rat *LASS1* (GenBank accession number: DQ479969) has a length of 1 053 bp and potentially encodes 350 amino acids as shown in Figure 2. Compared with the predicted ORF sequence (XM_224734), rat *LASS1* cDNA is short of 42 bp between the basepairs of 250th and 291st , and has an additional fragment of 39 bp before the termination codon (TGA), as shown in Figure 3. Sequence comparison showed that there are 92% identity between mouse *LASS1* mRNA and the cDNA sequence of rat *LASS1*, and 96% identity between the two deduced peptide sequences.

RPS-BLAST program revealed that the deduced amino acid sequence contains a Lag1p motif and a TLC domain of five predicted transmembrane alpha helices conserved in Lag1p, Tram, and Cln8 (SMART accession number SM0724) ER membrane associated proteins[25]. Figure 4 shows the comparison between the yeast Lag1p motif and the corresponding sequences in the homologs. The transmembrane prediction (TMHMM) revealed that the sequence of rat Lass1p

has five transmembrane domains at the amino acids 106～128, 143～165, 177～199, 237～259, 280～302 (Figure 5). An ER membrane retention signal (KKXX or KXKXX) [16] was detected at the C-terminal (KDKLF) (Figure 2).

```
                                    -41 CGGGTGCAAATAGCAGCGGAGACCGCGGACGAGAGGGTAGC
   1 ATGGCTGCTGCAGCGACGACCACCGGGCTCGAGGCGCCAGAGCCCATGCCGAGTTATGCAGAGATGTTGCAACGA   75
     M  A  A  A  A  T  T  T  G  L  E  A  P  E  P  M  P  S  Y  A  E  M  L  Q  R
  76 AGCTGGGCCTCGGCGCTGGCCGCAGCACGGGGCTGCGGGGACTGCGGCTGGGGACTGGCGCGCCGCGGCCTGGCG  150
     S  W  A  S  A  L  A  A  A  R  G  C  G  D  C  G  W  G  L  A  R  R  G  L  A
 151 GAGCACGCTCATCTGGCTGCACCCGAGCTGCTGCTGGCCGTGCTCTGCGCTCTGGGGTGGACAGCGTTGCGCTCA  225
     E  H  A  H  L  A  A  P  E  L  L  L  A  V  L  C  A  L  G  W  T  A  L  R  S
 226 GCAGCCACCACGCGCATCTTTCGGCCCCTGGCCAAGCGGTGTCGCCTGCAGCCTAGAGACGCGGCTAGGCTGCCT  300
     A  A  T  T  R  I  F  R  P  L  A  K  R  C  R  L  Q  P  R  D  A  A  R  L  P
 301 GAGAGTGCCTGGAAGCTTCTGTTCTACTTGGCCTGTTGGAGCTACTGTGCCTACCTGCTCTTGGGCACCAGTTAC  375
     E  S  A  W  K  L  L  F  Y  L  A  C  W  S  Y  C  A  Y  L  L  L  G  T  S  Y
 376 CCTTTCTTCCATGACCCGCCCTCTGTCTTCTACGGCTGGAGGTCGGGCATGGCAGTGCCCTGGGACATAGCGGTC  450
     P  F  F  H  D  P  P  S  V  F  Y  G  W  R  S  G  M  A  V  P  W  D  I  A  V
 451 GCCTACCTGCTGCAGGGGAGTTTCTACTGTCATTCCGTCTATGCCACCGTGTACATGGACAGCTGGCGCAAGGAC  525
     A  Y  L  L  Q  G  S  F  Y  C  H  S  V  Y  A  T  V  Y  M  D  S  W  R  K  D
 526 TCGGTGGTCATGCTGGTACACCATGTGGTCACCTTACTGCTCATCGCCTCTTCCTATGCGTTCCGATACCACAAC  600
     S  V  V  M  L  V  H  H  V  V  T  L  L  L  I  A  S  S  Y  A  F  R  Y  H  N
 601 GTGGGCCTCCTCGTGTTCTTCCTGCATGACGTCAGCGATGTGCAGCTGGAGTTCACCAAGCTCAACATCTACTTC  675
     V  G  L  L  V  F  F  L  H  D  V  S  D  V  Q  L  E  F  T  K  L  N  I  Y  F
 676 AAGGCCAGGGGTGGCGCCTACCATCGATTGCACGGGCTGGTGGCCAACCTGGGCTGCCTCAGCTTCTGCTTCTGC  750
     K  A  R  G  G  A  Y  H  R  L  H  G  L  V  A  N  L  G  C  L  S  F  C  F  C
 751 TGGTTCTGGTTCCGCCTCTACTGGTTCCCACTCAAGGTTCTCTATGCCACCTGGCACTGCAGCCTGCAGTCTGTG  825
     W  F  W  F  R  L  Y  W  F  P  L  K  V  L  Y  A  T  W  H  C  S  L  Q  S  V
 826 CCTGACATTCCGTACTATTTCTTCTTCAACACTCTGCTGCTGCTCCTCCTGGTCATGAACATCTACTGGTTCCTG  900
     P  D  I  P  Y  Y  F  F  F  N  T  L  L  L  L  L  L  V  M  N  I  Y  W  F  L
 901 TACATTGTGGCTTTTGCAGCCAAGGTGCTGACCGGTCAGATGCGTGAACTGGAAGACTTGCGGGAATACGACAGT  975
     Y  I  V  A  F  A  A  K  V  L  T  G  Q  M  R  E  L  E  D  L  R  E  Y  D  S
 976 CTGGAAGCGCAGACAGCCAAGCCCTGCAGAGCTGAGAAGCCTCTAAGGAATGGCCTGGTGAAGGACAAACTCTTC 1050
     L  E  A  Q  T  A  K  P  C  R  A  E  K  P  L  R  N  G  L  V  K  D  K  L  F
1051 TGAGTCTCTTGTCCTCAACTCCCGCCATCCAGGACTCTGTCCCGTCCTACCTGGGATACTGGCCCCGCCCCTGGA 1125
1126 GACTGGACCCAGTCCCCGGAGGTCTGCTCCCACCCCTGGAGGCCTGGCCTCGCCTTTGGCGGCATGGCCACGCCT 1200
1201 GCAGGACGATGGCCCCGCCCTAAGATGCAGGATGCCACCCTTCTCCAGGGACTCTGGCTGCCAGCAGCTCCGCCT 1275
1276 TTCAGGTCAATTCTCGACCACCCACCTTGGGACCCAGCCCAGTCCTGCCCTCTGGGTAAGTGGGGTCTGGACACG 1350
1351 CCCCCTCCAGGACCTCAAAGCACCCTCGACCTACGGTCACCAGCCCACTGGTCCTGGACGCAGTGGACTCTGCTG 1425
1426 ACTCTCTTGGACACCGCCTGGGAGGAAAATGCTCCCTGTCTGCCACCGTTTTTGCGACCACCTCCTCCTTCTGCT 1500
1501 CTTGCTGCCCTCAACGACCCTGGCCCACGCGCCAGCATCCTTGGGCCCCGCTGCCGCCCTGCTCCAGGTTCTCGG 1575
1576 GCTTCCCGAAACGCCCCGGAGCGTCCCCACACTCCGACCTGTGCCTCCTGTCATGTGGCGCCTATTCCGCAGCCG 1650
1651 CAACCCCCAGGAGGCCAGAGTGGGGCGCCCTCTGAAACCATGCCACGTGGAGGAACTGGGGGTTGCCGGAAACAT 1725
1726 TGTGCGCCACATCCCCGACAGCGGTCTGTCCTCCAGGATCATACAGCCCGCCAGAACCTCAGGGCTGTGCCCCGA 1800
1801 GTGGACCGTCGTCTTTGACCTGTCGAGCGTGGAGCCCATAGAGCGCCCAACACGCGCGCGCCTAGAGTTGCGGCT 1875
1876 GGAGGCTGAGAGCGAAGATACACGAGGGTGGGAGCTAAGCGTGGCGCTGTTGGCCGAGGCTGAGCATCCTGGGCC 1950
1951 TGAGCTACTGCGCGTGCAGGCGCCACCCGGGCTGCCACTGCGCGCAGACCTACTGGGGACTGCAGTAGCGGCTAA 2025
2026 CGCATCGGTGCCGCGCACTCTGCGCTTGGCGCTGGCGCTGCACCCTGGGGCCGCTGCAACCTGTGGGCGCCTGGC 2100
2101 TGAGGCCTCGTTGCTGCTGGTGACGCTGGACCCACGCCTGTGTCCCTTGCCGCGGTCACGGCGCCACACGGAGCC 2175
2176 CAGGGTGGGAGGTGGTCCAGTGGGCACATGTCGCACCCGACGGTTGCACGTGAGCTTCCGTGAGGTGGGCTGGCA 2250
2251 CCGCTGGGTGATTGCGCCGCGGGGCTTTCTAGCCAACTTCTGCCAGGGCACGTGTGCGCTACCTGAAACACTGAG 2325
2326 GGGACCCGGCGGGCCGCCTGCACTTAACCACGCTGTGCTGCGCGCGCTCATGCACGCAGCTGCTCCCACCCCGGG 2400
2401 TGTAGGCTCGCCCTGCTGCGTGCCAGAGCGTCTGTCGCCTATCTCCGTGCTCTTCTTCGATAATAGTGACAACGT 2475
2476 GGTGCTGCGCCACTACGAGGACATGGTGGTGGATGAGTGTGGCTGTCGCTGACCACCCAGGACACCCTTGCAGGG 2550
2551 ACGGCCCCACGCGAAAAGCAGGGACCGTTTGTTCATGTTTTATTGGTGACAAAAAGCTTAAAACAAATTTGACCA 2625
2626 AAAAAAAAAAAAAAAAAAAAAA 2648
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Fig. 2 The cDNA and deduced amino acid sequence of rat *LASS1*

(GenBank accession number: DQ479969). The start and stop codons are in gray background.

```
                                                                    250
LASS1 cDNA  ATCTTTCGG----------------------------------------CCCCTGGCC
            :::::::::                                       :::::::::
Predicted   ATCTTTCGGGTCAGTGAAACCTGGGGACCCCAGAGACTTGCGTGCGAGAGACCCCTGGCC
                    250       260       270       280       290       300

              1000      1010      1020      1030      1040      1050
LASS1 cDNA  AAGCCCTGCAGAGCTGAGAAGCCTCTAAGGAATGGCCTGGTGAAGGACAAACTCTTCTGA
            ::::::::::::::::::                                      :::
Predicted   AAGCCCTGCAGAGCTGAG----------------------------------------TGA
              1040      1050
```

Fig. 3　Comparison of the differences between the cDNA sequence and the predicted sequence (GenBank accession number: XM_224734) of rat *LASS1*

Name	Position	Lag1p motif	Identity	Similarity
Lag1Sc	246	RKDYKELVFHHIVTLLLIWSSYVFHFTKMGLAIYITMDVSDFFLSLSKTLNY		
Lac1Sc	246	RKDHNELTFHHIVTLLLIWSSYVFHFTKMGLPIYITMDVSDFLLSFSKTLNY	88.5%	88.5%
Lass1At	154	RSDFGVSMGHHITTLVLIVLSYICRLTRAGSVILALHDASDVFLEIGKMSKY	40.4%	55.8%
Lass1Tv	129	TKDIKMDIAHHFVTITMLTGCALVARPVGGLSIMLLHDWVDIFLYSGKVMNY	36.5%	50.0%
Lass1Hs	173	RKDSVVMLLHHVVTLTLIVSSYAFRYHNVGILVLFLHDISDVQLEFTKLNIY	40.4%	55.8%
Lass1Mm	173	RKDSVVMLVHHVVTLLLIASSYAFRYHNVGLLVFFLHDVSDVQLEFTKLNIY	46.2%	57.7%
Lass1Rn	173	RKDSVVMLVHHVVTLLLIASSYAFRYHNVGLLVFFLHDVSDVQLEFTKLNIY	46.2%	57.7%

Fig. 4　Comparison of the yeast Lag1 motif (Lag1Sc) with the corresponding sequences in the homologs

The percent identity and similarity with respect to Lag1Sc is tabulated. Lag1Sc, *Saccharomyces cerevisiae* Lag1p; Lac1Sc, *S. cerevisiae* Lac1p; Lag1At, *Arabidopsis thaliana* Lag1p; Lag1Tv, *Trichomonas vaginalis* Lag1p; Lass1Hs, *Homo sapiens* Lass1p; Lass1Mm, *Mus musculus* Lass1p; Lass1Rn, *Rattus norvegicus* Lass1p.

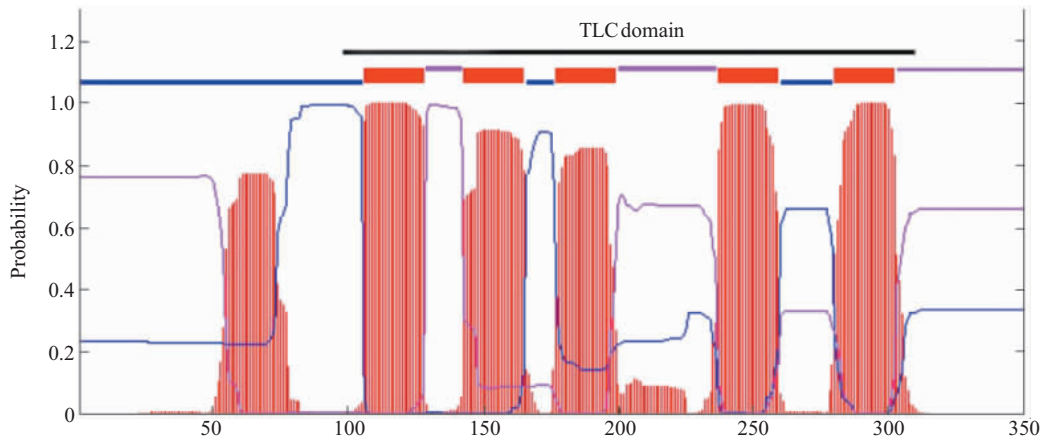

Fig. 5　The transmembrane domains of rat Lass1p, predicted using TMHMM server v.2.0

The TLC domain (TRAM, LAG1 and CLN8 homology domains) is indicated by a horizontal bar. ——: transmembrane; ——: inside; ——: outside.

2.3　Semi-quantitative RT-PCR analysis

We selected 18 cycles for 18 S rRNA and 22 cycles for *LASS1* as optimal PCR conditions after testing linear amplification range for each gene. PCR samples (10 μl each) were electrophoresed on 2.5% agarose gels as shown in Figure 6a. Average IDVs of both genes in each age-group were calculated respectively and its ratio of the *LASS1* divided by the 18S rRNA were plotted in Figure 6b. It's shown that the expression level of *LASS1* was lower in newborn rats than in adult rats (1～6 months) and reached the peak at the age of 6 month, then decreased as rats aging. The expression of *LASS1* was much lower at the age of 24 month than at the age of 6 month and 12 month.

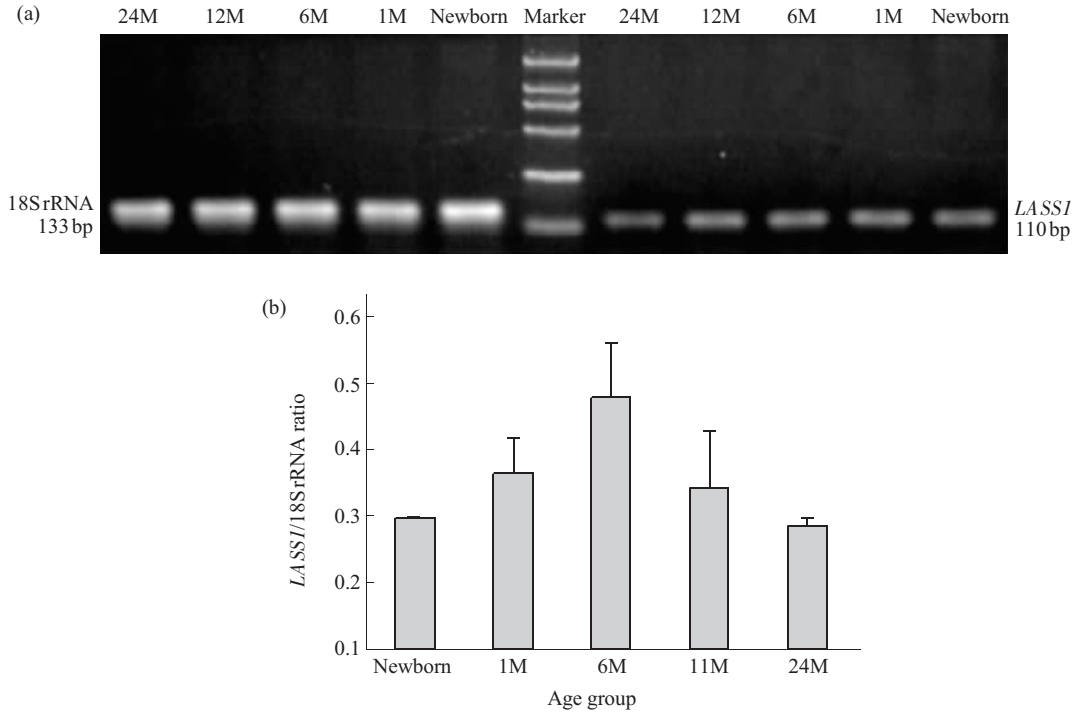

Fig. 6 Semi-quantitative RT-PCR analysis of *LASS1* in rat cerebral cortices

PCR samples (10 μl) of 18S rRNA (left, 133 bp, 18 cycles of amplification) and *LASS1* (right, 110 bp, 22 cycles of amplification) were electrophoresed on 2.5% agarose gels, stained with ethidium bromide (a). Age related *LASS1* expression (b) indicated by the ratio of *LASS1*/18S rRNA signal (integrated density of amplification in each groups, n=4).

2.4 Northern blot analysis

To further confirm our semi-quantitative findings, we performed Northern blot analyses of 30 μg total RNAs from rat cerebral cortex tissues at varying ages. *LASS1* was detected in rat brain as a band of approximately 2.7 kb. As shown in Figure 7, the expression of *LASS1* was up-regulated in young rats and down-regulated with aging.

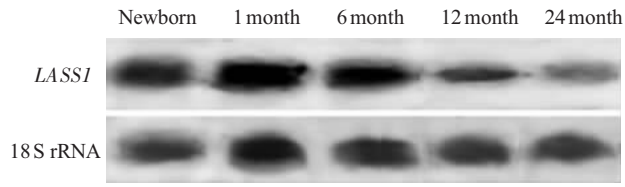

Fig. 7 Northern blot analysis of *LASS1* transcription

Total RNAs (30 μg each) from rat cerebral cortex tissues at varying ages were resolved on a 1.2% denaturing agarose gel, transferred to a nylon membrane and hybridized to a *LASS1* cDNA biotinylated labeled probe. The membrane was striped and re-hybridized to a 18 S rRNA probe.

3 Discussion

We have cloned and sequenced the cDNA of rat *LASS1*, which quite different to the previous predicted sequence in GenBank. The cDNA sequence of rat *LASS1* is quite similar to that of mouse and human, it contains two nonoverlapping open reading frames (ORF) of a conserved LASS1-GDF1 bicstronic transcript, and *LASS1* is the upstream ORF, as had been called mammalian upstream of growth and differentiation 1, *mUOG1*[4,15]. The ORF of rat *LASS1* has a length of 1 053 bp and potentially encodes 350 amino acids, which possesses five predicted transmembrane domains and an ER retention signal at the C-terminal end, shares the same Lag1p motif with mouse and a quite similar TLC domain with mouse and human.

In spite of that all Lag1p homologs are expected to be involved in ceramide/sphingolipid metabolism, it seems that the function of Lag1p/Lass1p is quite different to its homologues in the same organism. For example, both Lag1p and Lac1p are considered as essential components or subunits of the ceramide synthase. However, Lag1p provides the majority of ceramide synthase function though *LAC1* expression was approximately threefold higher than *LAG1*

expression in yeast *S. cerevisiae* [26]. Moreover, deletion [1] or moderate overexpression of *LAG1* resulted in increased longevity while higher expression curtailed life span, but deletion of *LAC1* had no effect[8]. In addition, *LASS1* mRNA has a limited tissue expression profile in mouse[4] and human[3], it is limited to brain, skeletal muscle, and testis. However, the *LASS1* homologues, *LASS2*, *LASS4*, *LASS5* and *LASS6* showed a distinct and broader expression profile [5,6]. As data have shown that in brain C18：0-ceramide is the major ceramide[22,23], while *LASS1* is expressed in brain abundantly and specifically [5], and Lass1p selectively regulates the synthesis of C18：0-containing sphingolipids in mammalian cells [15], suggesting *LASS1* may play a special role in neuron metabolism or aging process.

From newborn to 24 months, rat brain experiences a senescence process. SA-β-Gal staining has been considered as a biomarker for replicative senescence [24], but has not been used in neuron aging until now. In the present study, we found that the SA-β-Gal expression increased significantly in aged neurons of rat cerebral cortex. Although not all neurons in brain undergo exactly such changes, we still think SA-β-Gal may be considered as a sign of neuron senescence.

Many genes change expression with age in cortex of mouse and human, such as heightened expression of immune-related genes and depression of genes associated with learning and with metabolism, cell growth and division [28]. Age-related gene expression alterations may be a result of accumulated DNA damage[29], but the changed gene expression might play a role in the process of neuron aging. Here we found *LASS1* gene expression in rat cerebral cortex was up - regulated in adult rats (1～6 month) and down - regulated as the animal aging by semi-quantitative RT-PCR analysis and Northern blot. The result is similar to that of its homologue in the yeast. Northern blot analysis had revealed that in yeast *Saccharomyces cerevisiae* the *LAG1* transcript level had a small increase in early life span from 2- to 5-generation old cells, and then decreased with replicative age of yeast cells [1]. Whether age-related *LASS1* expression alternation has more significance in neuron aging needs further investigation.

LASS1 has been expected to be involved in mammalian senescence *via* the ceramide/sphingolipid metabolism pathway [27]. But there are still inconsistencies between *LAG1/LASS1* involved in ceramide synthsis and longevity. As Lag1p and Lac1p both provides C26：0-CoA-dependent ceramide synthase activity, but only *LAG1* has effect on the yeast life span. The present study demonstrated that *LASS1* expression declines during aging, thus C18：0 ceramide synthase activity should be decreased in rat cortex. However, previous data showed that long-chain ceramides were accumulated during normal brain aging and in the brains of Alzheimer's disease patients (C18：0 and C24 ceramides increased) [21]. Data also showed overexpression of *LAG1* and *LAC1* could not overproduce ceramide [12]. All these together suggest that *LAG1/LASS1* may be involved in cellular or organismal aging through an unknown or more complex mechanism.

References

1 D'mello N P, Childress A M, Franklin D S, *et al*. Cloning and characterization of *LAG1*, a longevity-assurance gene in yeast. J Biol Chem, 1994, **269** (22): 15451～15459

2 Brandwagt B F, Mesbah L A, Takken F L, *et al*. A longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternate f.* sp. lycopersici toxins and fuminosin B1. Proc Natl Acad Sci USA, 2000, **97** (9): 4961～4966

3 Jiang J C, Kirchman P A, Zagulski M, *et al*. Homologs of the yeast longevity gene *LAG1* in *Caenorhabditis elegans* and human. Genome Res, 1998, **8** (12): 1259～1272

4 Lee S J. Expression of growth/differentiation factor 1 in the nervous system: conservation of a bicistronic structure. Proc Natl Acad Sci USA, 1991, **88** (10): 4250～4254

5 Mizutani Y, Kihara A, Igarashi Y. Mammalian Lass6 and its related family members regulate synthesis of specific ceramides. Biochem J, 2005, **390** (Pt 1): 263～271

6 Pan H, Qin WX, Huo K K, *et al*. Cloning, mapping, and characterization of a human homologue of the yeast longevity assurance gene *LAG1*. Genomics, 2001, **77** (1-2): 58～64

7 Egilmez N K, Chen J B, Jazwinski S M. Specific alterations in transcript prevalence during the yeast life span. J Biol Chem, 1989, **264** (24): 14312～14317

8 Jiang J C, Kirchman P A, Allen M, *et al*. Suppressor analysis points to the subtle role of the *LAG1* ceramide synthase gene in determining yeast longevity. Exp Gerontol, 2004, **39** (7): 999～1009.

9 Barz W P, Walter P. Two endoplasmic reticulum (ER) membrane proteins that facilitate ER-to-Golgi transport of glycosylphosphatidylinositol-anchored proteins. Mol Biol Cell, 1999, **10** (4): 1043～1059

10 Horvath A, Sutterlin C, Manning-Krieg U, *et al*. Cermide synthesis enhances transport of GPI-anchored proteins to the Golgi apparatus in yeast. EMBO J, 1994, **13** (16): 3687～3695

11 Guillas I, Kirchman P A, Chuard R, *et al*. C26-CoA-dependent ceramide synthesis of *Saccharomyces cerevisiae* is operated by Lag1p and Lac1p. EMBO J, 2001, **20** (11): 2655～2665
12 Schorling S, Vallee B, Barz W P, *et al*. Lag1p and Lac1p are essential for the Acyl-CoA-dependent ceramide synthase reaction in *Saccharomyces cerevisiae*. Mol Biol Cell, 2001, **12**(11): 3417～3427
13 Vallee B, Riezman H. Lip1p: a novel subunit of acyl-CoA ceramide synthase. EMBO J, 2005, **24** (4): 730～741
14 Riebeling C, Allegood J C, Wang E, *et al*. Two mammalian longevity assurance gene (*LAG1*) family members, trh1 and trh4, regulate dihydroceramide synthesis using different fatty acyl-CoA donors. J Biol Chem, 2003, **278** (44): 43452～43459
15 Venkataraman K, Riebeling C, Bodennec J, *et al*. Upstream of growth and differentiation factor 1 (*uog1*), a mammalian homolog of the yeast longevity assurance gene 1 (*LAG1*), regulates N-stearoylsphinganine (C18- (dihydro)ceramide) synthesis in a fumonisin B1-independent manner in mammalian cells. J Biol Chem, 2002, **277** (38): 35642～35649
16 Guillas I, Jiang J C, Vionnet C, *et al*. Human homologues of *LAG1* reconstitute CoA-dependent ceramide synthesis in yeast. J Biol Chem, 2003, **278** (39): 37083～37091
17 Perry D K, Hannun Y A. The role of ceramide in cell signaling. Biochim Biophys Acta, 1998, **1436** (1～2): 233～243
18 Alessenko A V. The role of sphingomyelin cycle metabolites in transduction of signals of cell proliferation, differentiation and death. Membr Cell Biol, 2000, **13** (2): 303～320
19 Gulbins E. Regulation of death receptor signaling and apoptosis by ceramide. Pharmacol Res, 2003, **47** (5): 393～399
20 Yu Z F, Nikolova-Karakashian M, Zhou D, *et al*. Pivotal role for acidic sphingomyelinase in cerebral ischemia-induced ceramide and cytokine production, and neuronal apoptosis. J Mol Neurosci, 2000, **15** (2): 85～97
21 Cutler R G, Kelly J, Storie K, *et al*. Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. Proc Natl Acad Sci USA, 2004, **101** (7): 2070～2075
22 O'Brien J S, Rouser G. The fatty acid composition of brain sphingolipids: sphingomyelin, ceramide, cerebroside, and cerebroside sulfate. J Lipid Res, 1964, **5** (3): 339～342
23 Pettus B J, Baes M, Busman M, *et al*. Mass spectrometric analysis of ceramide perturbations in brain and fibroblasts of mice and human patients with peroxisomal disorders. Rapid Commun Mass Spectrom, 2004, **18** (14): 1569～1574
24 Dimri G P, Lee X, Basile G, *et al*. A biomarker that identifies senescent human cells in culture and in aging skin *in vivo*. Proc Natl Acad Sci USA, 1995, **92** (20): 9363～9367
25 Winter E, Ponting C P. TRAM, LAG1 and CLN8: members of a novel family of lipid-sensing domains? Trends Biochem Sci, 2002, **27** (8): 381～383
26 Kolaczkowski M, Kolaczkowska A, Gaigg B, *et al*. Differential regulation of ceramide synthase components *LAC1* and *LAG1* in *Saccharomyces cerevisiae*. Eukaryot Cell, 2004, **3** (4): 880～892
27 Obeid L M, Hannun Y A. Ceramide, stress, and a "LAG" in aging. Sci Aging Knowledge Environ, 2003, **2003** (39): PE27
28 Sharman E H, Sharman K G, Bondy S C. Parallel changes in gene expression in aged human and mouse cortex. Neurosci Lett, 2005, **390** (1): 4～8
29 Lu T, Pan Y, Kao S Y, *et al*. Gene regulation and DNA damage in the ageing human brain. Nature, 2004, **429** (6994): 883～891

LASS1 基因克隆及其在大鼠脑皮层的表达与神经元衰老的相关性初步研究

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摘要 长寿保障基因 *LAG1* 是从酵母中克隆的与酵母寿命相关的基因，随酵母生命衰老而表达发生变化. 对大鼠中同源基因 *LASS1* 进行克隆、测序和序列分析，发现其 mRNA 序列不同于 GenBank 中的预测序列，开放阅读框包含 1 053 碱基对，编码蛋白由 350 个氨基酸组成，内含 Lag1 蛋白家族保守的 Lag1p motif 和 TLC 结构域. 从新生、1 月龄、6 月龄、12 月龄和 24 月龄大鼠脑顶叶皮质提取总 RNA，用半定量 RT-PCR 及 RNA 印迹方法对 *LASS1* 在大鼠脑皮质中的表达随年龄变化情况进行分析. 结果表明，出生后 *LASS1* 表达量随年龄增加而增高，至 6 月龄达高峰，然后随年龄增加而逐渐下降，至 24 月老龄鼠达最低. 衰老相关 β 半乳糖苷酶(SA-β-gal)对鼠脑皮层染色发现，神经元阳性染色随年龄增长明显增加. 大鼠 *LASS1* 基因表达在正常衰老过程中发生变化，为进一步研究该基因的作用奠定了基础.

关键词 *LASS1* 基因克隆，大鼠，基因表达，神经元衰老

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