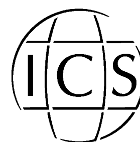
ARTICLE IN PRESS

International Congress Series xx (2003) xxx–xxx



www.ics-elsevier.com

Seeking for ageing-associated gene expression in cerebral tissue of senescence-accelerated mouse (SAM)

Chong Zhang [a,b], Jinyan Cheng [a], Qingxuan Chen [a,*]

[a] *Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100080, China*
[b] *Graduate School of Chinese Academy of Sciences. Beijing 100039, China*

Received 11 June 2003; received in revised form 19 June 2003; accepted 10 September 2003

UNCORRECTED PROOF

Abstract. In this study, the ageing-specific expression genes of the murine cerebrum were investigated by employing differential-display reverse transcription polymerase chain reaction (DDRT-PCR) in three senescence-accelerated mouse (SAM) strains: SAMP8/Ta, SAMP10/Ta and SAMR1TA. In the comparison of gene expression profile in different strain mice, 16 differential fragments have been detected, 3, 6 and 7 of them belong to SAMP10/Ta, SAMP8/Ta and SAMR1TA, respectively. Sequencing data indicated that those fragments are homologous with heat shock protein cognate protein 70, ATP-dependent mitochondrial RNA helicase, Dleu2 mRNA, Mouse DNA sequence from clone RP23-334C3 on chromosome X, ubiquinol-cytochrome *c* reductase complex (7.2 kDa), 60S ribosomal protein L21, FIS, phenylalkylamine Ca^{2+} antagonist (emopamil) binding protein, fucosyltransferase 9, glial cell line derived neurotrophic factor family receptor alpha 1, endonuclease/reverse transcriptase, PER1 interacting protein of the suprachiamatic nucleus homolog, centrosomal protein CG-NAP, ferritin heavy chain gene, NID-2 gene, and prkdc gene for DNA-dependent protein kinase catalytic subunit. © 2003 Published by Elsevier B.V.

Keywords: Senescence-accelerated mouse; Ageing-associated gene expression; DDRT-PCR; Cerebral tissue

Senescence-accelerated mouse (SAM), which has been established as a murine model of the SAM strain, is actually a group of related inbred strains including nine strains of accelerated senescence-prone, short-lived mice (SAMP), and three strains of accelerated senescence-resistant, long-lived mice (SAMR) [1,2]. SAMP-strain mice show relatively strain-specific ageing-associated phenotypic pathologies such as a shortened life span and early manifestation of senescence. In this study, three strain mice were applied to seek for ageing-associated expression genes: SAMP8/Ta strain mice have short life span and ageing-related deficits in learning and cognitive abilities, emotional disorder, abnormal

* Corresponding address. Tel.: +86-10-6255-3160; fax: +86-10-6255-1951.
E-mail address: qingxuanchen@yahoo.com (Q. Chen).

0531-5131/ © 2003 Published by Elsevier B.V.
doi:10.1016/S0531-5131(03)01683-2

ARTICLE IN PRESS

circadian rhythms and impaired immune response [3]. SAMP10/Ta strain mice have short life span and ageing-related deficits in learning and memory, emotional disorder and abnormal circadian rhythms. SAMR1TA strain mice have normal life span and no ageing-related disorders as control.

The model animal SAM exhibiting senescence-accelerated phenotype indicates that senescence is, at least to some extent, controlled genetically. To date, SAM mouse had been widely used in researching ageing-associated diseases. However, few are involved in that what cause SAMP mouse shortened life span on molecular level [4–6].

1. Materials and methods

1.1. Materials

Animals and Feed Condition: senescence-accelerated mice (SAM): SAMR1TA, SAMP8/Ta, SAMP10/Ta were bought from Kyoto University, Japan. The mice were housed in the experimental animal center of our Institute under the same condition in a clean facility on a 12-h light/dark cycle, and were given a standard commercial pellet diet and tap water ad libitum. The standard commercial pellet diet used in this experimental animal is the commercial forage for propagation mice and was bought from the feed-processing plant of the experimental animal center, the academy of military medicine sciences.

Primers are synthesized by SBS, Beijing. Primer sequences are as follow:

A1: 5′ -ACAGA GCACA; APG: 5′ -AAGCT TTTTT TTTTT TG; APC: 5′ -AAGCT TTTTT TTTTT TC; APA: 5′ -AAGCT TTTTT TTTTT TA.

1.2. Methods

1.2.1. Total RNA isolation

The male mice used for the experiment were decapitated, and the cerebral tissues were taken out. Cut out about 30-mg cerebral tissue of each mouse, then according to the RNeasy mini-Kit protocol, total RNA was isolated. The total RNA samples are treated with RQ1 RNase-Free DNase and stored at −20 °C before use.

1.2.2. Reverse transcription of the RNA and PRC amplification

Three PCR tubes were used for each sample. In each tube, the reagents were added as follows: 2 μl of total RNA (1 μg), 2 μl of 10× RT buffer, 1.6 μl of 2.5 mM dNTPs, 2 μl of 3′ Primer, 0.5 μl of RNasin Ribonuclease Inhibitor (40 iu/μl), add double-distilled water to 19 μl. 65 °C for 5 min, 42 °C for 10 min, then in each tube add 1 μl of M-MuLV Reverse Transcriptase (200 iu); 37 °C for 50 min, 75 °C for 5 min. The RT products were amplified with PCR system.

1.2.3. Identification and collection of DD bands

The PCR products above were used for 6% Urea denature polyacrylamide gel electrophoresis and the electrophored gel was exposed to double X-ray films for 72 h at −20 °C. The differential display bands (DD bands) were located on the film and then cut out the interesting DD bands from the gel and put each band into a 1.5-ml tube. The DD fragment was extracted from its correspondence gel.

UNCORRECTED PROOF

ARTICLE IN PRESS

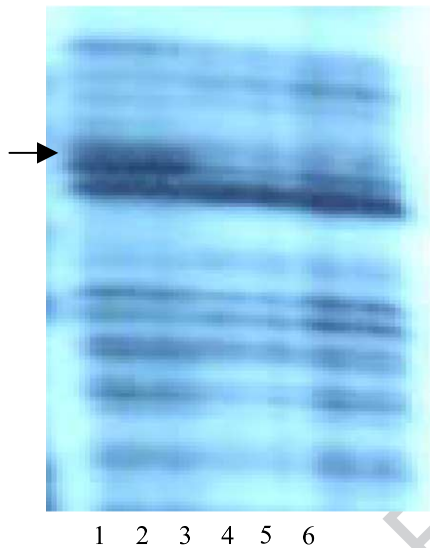


Fig. 1. Comparison of gene expressions among differential age SAMP8/Ta using DDRT-PCR. The arrow points the differential display band. The numbers below the figure represent as follow: 1, 2 samples come from 11-month SAMP8/Ta; 3, 4 samples come from 4-month SAMP8/Ta; 5, 6 samples come from 2-month SAMP8/ Ta.

78

1.2.4. Ligation and transformation of the purified products 79

Ligate the purified products into pGEM-T Easy Vector according to the protocol of the 80
manual. Then the ligated products are transformed into *Escherichia coli* DH.5 α competent 81

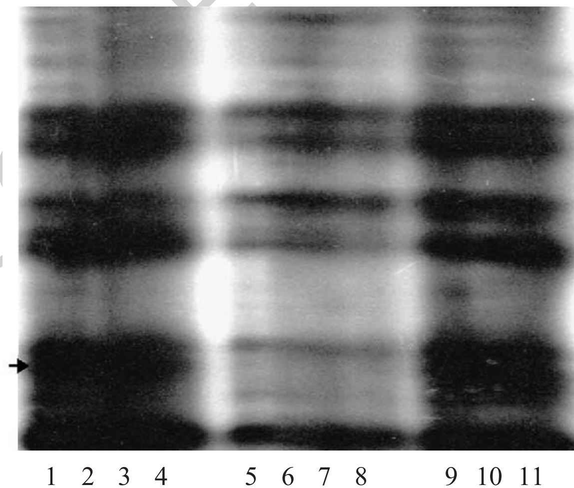


Fig. 2. Comparison of gene expression among differential strains SAM mouse. The arrow points the differential gene expression band. The numbers below the figure represent as follow: 1, 2, 3, 4 samples come from SAMP10/ Ta and aged 18, 12, 4, and 2 month, respectively; 5, 6, 7, 8 samples come from SAMR1TA and aged 18, 11.5, 4, and 2 month, respectively; 9, 10, 11 samples come from SAMP8/Ta and aged 11, 4, 2 month, respectively.

UNCORRECTED PROOF

ARTICLE IN PRESS

t1.1 Table 1
t1.2 Features of sequenced clone and results of BLAST search

Clone	Size (bp)[a]	DNA homology (BLAST)	Accession no.	Identity (%)	Overlap
1[b]	120	*Mus musculus* heat shock cognate protein 70	BC006722.1	99	104
2	132	*Mus musculus* similar to suppressor of var1, 3-like 1	AK031911.1	94	142
S. cerevisiae [Homo sapiens]					
3	166	Mouse DNA sequence from clone RP23-334C3 on chromosome	XAL672057.8	100	132
4	181	*Mus musculus* Dleu2 mRNA	AF380423	98	191
7	256	*Mus musculus* similar to ubiquinol-cytochrome *c* reductase complex (7.2 kDa)	BC024518.1	99	263
12	227	*Mus musculus* similar to 60S ribosomal protein L21	XM_193408.1	99	235
13	198	*Mus musculus* similar to FIS	AK003875.1	100	206
14	198	*Mus musculus* similar to FIS	AK003875.1	100	206
16	135	*Mus musculus* phenylalkyl-amine Ca^{2+} antagonist (emopamil) binding protein	BC004703.1	100	143
19	132	*Mus musculus* fucosyl-transferase 9	AK047650.1	94	136
20	103	*Mus musculus*, similar to glial cell line derived neurotrophic factor family receptor alpha 1	BC040251.1	100	73
24	255	*Mus musculus*, similar to ubiquinol-cytochrome *c* reductase complex (7.2 kDa)	BC024518.1	100	263
25	112	*Mus musculus*, similar to endonuclease/ reverse transcriptase	XM_207118.2	94	121
27	111	*Mus musculus*, PER1 interacting protein of the Suprachiamatic nucleus Homolog [*Rattus norvegicus*]	AK050919.1	97	122
29	108	*Rattus norvegicus* centrosomal protein CG-NAP	AB071391.1	91	116
32	98	*Mus musculus* similar to 60S ribosomal protein L21	XM_122501.1	100	105
33	98	*Mus musculus* similar to 60S ribosomal protein L21	XM_122501.1	99	105
36	177	Mouse ferritin heavy chain gene	M60170.1	100	184
40	184	*Mus musculus* partial NID-2 gene	MMU428512	99	195
41	145	*Mus musculus* prkdc gene for DNA-dependent protein kinase catalytic subunit	AB030754.1	93	48

t1.26 [a] The size does not include the primer sequences.

[b] The background of these fragments is as follow: Fragments come from differential age cerebral tissue in single strain. Clone 1, 12 month of SAMP10/Ta, clone 2, 11 month of SAMP8/Ta, clone 3, 2 month of SAMP8/Ta, clone 4, 4 month of SAMP10/Ta. Fragments come from differential strains of their cerebral tissue. Clone 7, SAMR1TA, clone 12, SAMP10/Ta, clone 13, SAMR1TA, clone 14, SAMP8/Ta, clone 16, SAMR1TA, clone 19, SAMP8/Ta, clone 20, SAMP10/Ta, clone 24, SAMP8/Ta, clone 25, SAMP10/Ta, clone 27, SAMP8/Ta, clone 29, SAMP8/Ta, clone 32, SAMR1TA, clone 33, SAMP8/Ta, clone 36, SAMR1TA, clone 40, SAMR1TA, and clone
t1.27 41, SAMR1TA.

UNCORRECTED PROOF

ARTICLE IN PRESS

cell, cultured in SOC medium at 37 °C overnight. The overnight-cultured products are used to purified plasmid DNA with UltraPureTMplasmid DNA mini-purification kit according to the manual protocol. The purified products are identified by PCR with the same primers of the insert fragments, and electrophored on 1.2% agarose gel.

1.2.5. DNA sequencing and its analysis

The DNA sequencing of differential fragment is finished by Sangon and Bioasia, and the data are compared in GenBank.

2. Results

Using DDRT-PCR method, the cerebral gene expressions at differential age were compared in the same strains of the SAMP8/Ta (age: 2, 3.5, 11 months), SAMP10/Ta (age: 2, 4, 12, 18.5 months) and SAMR1TA (age: 2, 4, 12, 18 months). Four differential bands had been found, which belong to 12 months of SAMP10/Ta (1), 11 months of SAMP8/Ta (2), 2 months of SAMP8/Ta (3), and 4 months of SAMP10/Ta (4). In addition, the differential strain mouse cerebral gene expressions were compared in the three strains. Forty-two differential bands had been located. A part of the result of the DDRT-PCR is shown in Figs. 1 and 2.

Of these differential bands, 20 had been reamplified and sequenced. The sequenced results had been analyzed on homology by using BLAST program to search NCBI-nr. The results are shown in Table 1.

3. Discussion

The investigation reported here demonstrated that employing DDRT-PCR to insight the differential gene expression of cerebral tissue among differential strains and vary age SAM mice can provide valuable data. Using the DDRT-PCR, we had found out 4 fragments that are ageing-associated expression. And 16 fragments that are strain-specific expression. The 4 ageing-associated expression fragments are homologous with heat shock protein cognate protein 70, ATP-dependent mitochondria RNA helicase, Dleu2 mRNA, and Mouse DNA sequence from clone RP23-334C3 on chromosome X, respectively.

4. Conclusion

In this investigation, the DDRT-PCR was used to compare the differential gene expression among differential age SAM mouse and between train of SAMP8/Ta, SAMP10/Ta and SAMR1TA. Twenty cDNA fragments were cloned which involved 15 known genes and 1 novel gene, most of the fragments reported are age-associated. The data obtained in these studies indicate that genes control the senile and accelerated senescence phenotypic pathologies in SAM mouse.

Acknowledgements

This project was supported by State Basic Research Developmental Program (No. G2000016107), and partly supported by Beijing Lingtai Bicheng Medical Technology.

UNCORRECTED PROOF

ARTICLE IN PRESS

References 121

[1] M.A. Lynch, Age-related impairment in long-term potentiation in hippocampus; a role for the cytokine, interleukin-1 β, Prog. Neurobiol. 56 (1998) 571–589. 122 123
[2] T. Takeda, M. Hosokawa, S. Takeshita, M. Irino, K. Higuchi, T. Matsushita, Y. Tomita, K. Yasuhira, H. Hamamoto, K. Shimizu, M. Ishii, T. Yamamuro, A new murine model of accelerated senescence, Mech. Ageing Dev. 17 (2) (1981) 183–194. 124 125 126
[3] T. Takeda, M. Hosokawa, K. Higuchi, Senescence-accelerated mouse (SAM): a novel murine model of accelerated senescence, J Am. Geriatr. Soc. 39 (9) (1991) 911–919. 127 128
[4] T. Takeda, Senescence-accelerated mouse (SAM): a biogerntological resource in aging research, Neurobiol. Aging 20 (1999) 105–110. 129 130
[5] N. Matsukawa, I. Tooyama, H. Kimura, T. Yamamoto, Y. Tsugu, Y. Oomura, K. Ojika, Increased expression of hippocampal cholinergic neurostimulating peptide-related components and their messenger RNAs in the hippocampus of aged senescence-accelerated mice, Neuroscience 88 (1) (1999) 79–92. 131 132 133
[6] J. Wang, T. Matsushita, K. Kogishi, C. Xia, A. Ohta, T. Chiba, A. Nakamura, H. Kondo, M. Mori, M. Hosokawa, K. Higuchi, Wild type ApoA-II gene does not rescue senescence-accelerated mouse (SAMP1) from short life span and accelerated mortality, J. Gerontol., Ser. A, Biol. Sci. Med. Sci. 55 (9) (2000) B432–B439. 134 135 136 137

UNCORRECTED PROOF

138