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Parallel changes in gene expression in aged human and mouse cortex

Edward H. Sharman *, Kaizhi G. Sharman, Stephen C. Bondy

Center for Occupational and Environmental Health, Department of Community and Environmental Medicine, University of California, 19772 Jamboree, Irvine, CA 92697-1825, USA

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Abstract

The intensity of expression of over 20,000 genes and expressed sequence tags within the cerebral cortex has previously been described for both the human and mouse genomes. In both these species, the degree of expression of a relatively limited number of cortical genes, around 300, is significantly altered during senescence. The extent of similarity between age-related alterations of levels of specific mRNAs in either species has been compared. There is a significant correlation between species in those genes whose expression changes markedly in either direction with aging. This parallel serves to validate the use of mouse strains to study general age-related genetic events associated with aging.

Keywords: Aging; Gene profile

The study of human aging often involves the use of animal models. In this context, there is a widespread use of various mouse strains. This species has especial utility due to the relative ease of genetic manipulation allowing production of animals with specific genes deleted or inserted. The rather short life span of mice also adds to their utility in aging research. This latter feature allows the administration of dietary supplements over a significant fraction of the life of a mouse, in attempts to retard the onset of adverse neurological events associated with senescence [14,15]. Consequently, it is important to establish the extent of similarity between genetic changes taking place with aging, in the human and the mouse brain.

In this report we have compared our results concerning age-effected changes in cortical mRNA levels in the CB6F1 mouse, with those reported for 39 humans, aged 13–79 years [4]. A good degree of correlation has been obtained with both genes whose expression decreases with age and those where an increase is apparent. The most notable correspondence is found in those mRNAs whose levels diminish with age.

Male CB6F1mice, a hybrid between C57BL/6JM and BALB/cJF from Harlan Labs (Indianapolis, IN), aged 4 months (young group) and 27 months (old group), were maintained on a 12 h light/dark cycle in a temperature controlled ($22 \pm 1\,^{\circ}C$) room. The CB6F1 hybrid was used in order to take advantage of both the genetic and phenotypic uniformity and the vigor – increased disease resistance, better survival under stress and greater natural longevity – typical of hybrids, while maintaining genetic similarity to the published C57BL/6 mouse genome sequence [19].

Food and water were provided ad libitum. Animals were fed a pelleted minimal basal diet (AIN-93M, Dyets #100900, Dyets Inc., Bethlehem, PA) consisting of 10% sucrose and 14% casein (w/w) as well as a minimal salt and vitamin mix. Separately-caged sentinel animals were maintained in the same room to monitor for the occurrence of infectious disease; no disease was detected during the treatment period. All experiments conformed to the National Institute of Health guide for the care and use of laboratory animals.

Mice were killed by cervical dislocation between 1040 and 1440 h and were visually inspected for signs of disease or other pathology. Whole brain was excised quickly; cerebellum and brain stem were removed and the remaining tissue was immediately frozen on dry ice and stored at

* Corresponding author. Tel.: +1 949 824 4769; fax: +1 949 824 2070.
E-mail address: esharman@uci.edu (E.H. Sharman).

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−70 °C. Total RNA was extracted using the TRI Reagent® kit (Molecular Research Center Inc., Cincinnati, OH), following the manufacturer's protocol. Aliquots of the total RNA were further purified on an RNeasy column (Qiagen Inc., Valencia, CA) to yield a 260–280 nm absorbance ratio of ≥1.9. RNA concentrations were determined by absorption at 260-nm wavelength.

Quantitative (real-time) polymerase chain reactions (qRT-PCR) were carried out on a LightCycler Instrument (Roche Diagnostics, Indianapolis, IN) using the QuantiTect SYBR Green RT-PCR reagent kit (Qiagen, Valencia, CA) according to the manufacturers' directions. Product fluorescence was detected at the end of the elongation cycle at 72 °C, except for Anxa4, for which a detection temperature of 77 °C was required. Expression levels for each sample were calculated as the average of two measurements, relative to β-actin expression.

A 20 μg aliquot of total RNA from each of three samples per treatment group was processed and applied to a Mouse Genome 430 2.0 GeneArray® chip according to the manufacturer's protocol (Affymetrix, Santa Clara, CA). The resulting dataset of 45101 messenger (mRNA) probeset expression levels contained in the DAT file for each sample was separately analyzed in DChip [8] and GeneSpring (Silicon Genetics, Redwood City, CA). The Robust Multi-array Average (RMA) method [5] in GeneSpring was used for normalization and for the estimation of data quality, enabling reliable inter-chip comparisons of probe expression changes. Expression fold-change was calculated as the power-2 exponential of the $\log_2$ difference.

Out of a total 45,101 probesets on each gene chip, RMA analysis yielded a subset of 14,719 probesets that were reliably ($p < 0.05$) expressed on all gene chips. Probesets whose expression differed between old and young sets of mice were collated using the criteria and procedures described above. From this analysis, expression levels of 412 probesets out of 14,719 were found to be significantly altered with age by 1.3-fold or greater. These were compared with the 587 human BA9 and BA47 cortical probesets found by Erraji-Benchekroun et al. [4] to change with age using lists of orthologous probesets downloaded from http://www.affymetrix.com. Inspection of these lists revealed that not all probesets in one species had orthologs in the other, and that some probesets were identified with multiple orthologous probesets in the other species. Analysis yielded a set of 499 mouse probesets orthologous to the 587 human age-responsive probesets and a set of 595 human probesets orthologous to the 412 age-responsive mouse probesets. A total of 29 genes that changed with age in both mice and humans were common to these two sets (Table 1), leaving 471 human probesets that had no age-responsive orthologs in mouse and 253 mouse probesets with no age-responsive probesets in human. Expression levels of five of the 29 common age-responsive genes were checked by qRT-PCR; in each case, the old-animal qRT-PCR levels differed significantly ($p < 0.05$) from the young-animal levels, and the direction of change agreed with the microarray findings (Table 1).

Among the 29 genes was that for glial-fibrillary acidic protein (GFAP). GFAP levels reflecting astrocytic response are known to be elevated in animals exposed to a variety of neurotoxic agents [10], and also with aging in both animals and humans [2,20]. In addition, a further increase in levels of this protein is found in age-related neurodegenerative disorders including Alzheimer's disease [12] and other dementias [6]. A significant proportion of common genes was related to acute phase inflammatory responses, including three complement proteins.

The levels of several mRNAs were greatly reduced in both aging mouse and human brain. These included those coding for proteins related to growth and division such as early growth response factor and cyclin D2. Other genes severely down-regulated with age in both species, were associated with regulation of metabolic activity. These include those for iodothyronine deiodinase and corticotropin releasing hormone, CRH.

A correlation between changes in age-induced expression of human and mouse genes was found with $R = 0.668$ (Fig. 1) and this was highly statistically significant ($p < 0.0001$).

This comparative study revealed an age-sensitive pattern of altered expression of several critical genes that was common to both species. This shared profile included age-related heightened expression of immune-related genes, together with depression of genes associated with learning and with metabolism, cell growth and division. This overlap is greater than it may appear since only 1006 unique Affymetrix probe sets have been identified that are common to mouse and man, despite the estimate of around 80% gene identity between the species [19].

Annexin A4 is a calcium- and phosphatidic acid-binding protein of uncertain function found in a number of mammalian brain regions. Its mRNA expression increases with age in both mice and humans. Annexin A4 protein expression is substantially greater in reactive than in quiescent astrocytes [3], suggesting it may play a role in neuroinflammation. Rat glioma cells that overexpress annexin A4 are more sensitive to ethanol cytotoxicity [11]. Thus seeking a possible connection between increased age-related levels of annexin A4 and the neurodegeneration associated with normal aging may be fruitful.

In B6C3F1 mouse that we have previously examined there was also a clear age-associated elevation of mRNAs coding for proteins associated with immune and inflammatory events. Out of the 21 genes whose expression was most markedly up-regulated with senescence, three were immunoglobulins and four were complement proteins [13]. This trend is paralleled in the aged C57BL/6 mouse [7]. Some of the differences between the human and mouse gene profile pertain to mRNAs coding for complement. All three complement transcripts C1qa, C3 and C4 were elevated in the mouse with aging; these are also ele-

Table 1
Genes common to human and mouse whose expression is significantly altered during senescence

Gene name	Human gene ID	Mouse gene ID	Old/young ratio		
			Human μArray	Mouse μArray	Mouse qRT-PCR
Sox4	6659	20677	0.58	0.67	
Arc	23237	11838	0.60	0.33	0.23
Complement C3	718	12266	0.62	1.43	
Arpp21	10777	19050	0.68	0.84	
Tribbles homolog 2	28951	217410	0.72	0.66	
Trophoblast glycoprotein	7162	21983	0.74	0.73	
Tyrobp	7305	22177	0.74	1.43	
Corticotropin releasing hormone	1392	12918	0.74	0.62	
Complement C1qa	712	12259	0.74	1.60	3.12
Cyclin D2	894	12444	0.77	0.64	
Deiodinase, iodothyronine, type II	1734	13371	0.79	0.67	0.64
Early growth response 4	1961	13656	0.79	0.76	
Dual specificity phosphatase 14	11072	56405	0.80	0.71	
Rrp22	10633	75668	0.81	0.68	
Kcnf1	3754	382571	0.81	0.70	
Dnajb5	25822	56323	0.81	0.69	
Dyrk2	8445	69181	0.83	0.75	
Fcer1g	2207	14127	0.83	1.37	
Gp1bb	2812	14724	0.83	0.77	
Histone deacetylase 9	9734	79221	0.86	0.74	
Cytochrome b-245, α polypeptide	1535	13057	0.90	1.36	
Myelin protein zero-like 1	9019	68481	0.95	0.71	
Zbtb16	7704	102538	1.12	1.33	
Spectrin beta 2	6711	20742	1.16	1.34	
Kinectin 1	3895	16709	1.18	1.14	
Annexin A4	307	11746	1.28	1.33	2.13
Zinc finger homeobox 1B	9839	24136	1.41	1.35	
Complement C4	720, 721	12268	1.50	2.68	7.75
Glial fibrillary acidic protein	2670	14580	1.82	2.09	

Mouse microarray (μArray) old/young animal ratios were calculated from data obtained from Affymetrix MG430 arrays and confirmed by real-time PCR (qRT-PCR); human ratios were calculated from microarray data as reported by Erraji-Benchekroun et al. [4]. Gene IDs correspond to those in the NCBI Gene database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene).

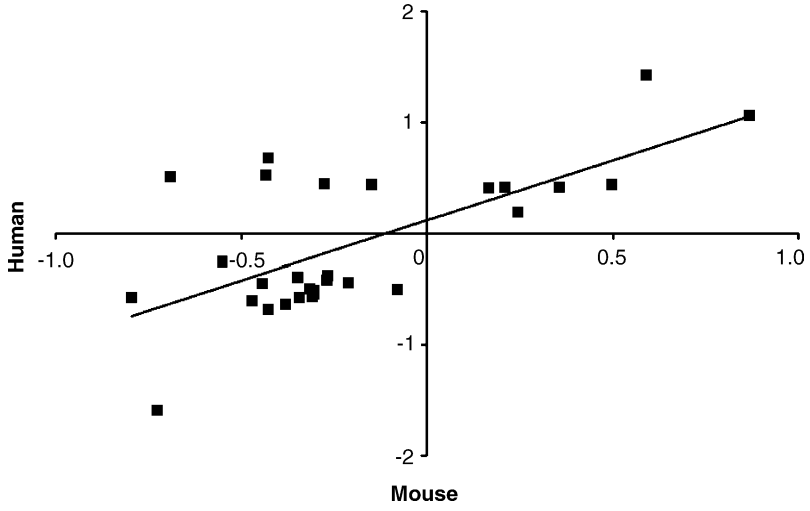

Fig. 1. Correlation of age-related changes in mouse and human cortex. Comparison of the extent and direction of change in genes common to human and mouse, whose expression levels are significantly up- or down-regulated during aging. Each point represents a single gene and numbers represent $\log_2$ of ratio-change of selected gene occurring with aging. A best-fit correlation between magnitude of changed expression in either species occurring with aging yielded a correlation coefficient R of 0.67 ($p < 0.0001$).

vated in temporal cortex of human Alzheimers patients [16]. However, in the normal human cortex, only complement C4 was significantly raised with age while complement C1qa and C3 were depressed. Thus, while inflammation and inappropriate immune responses are increasingly thought to be major events associated with brain aging, the use of the mouse to model human brain aging may have limitations. The evidence for elevation of complement transcripts in aged mouse brain [18] appears to be stronger than that for proteins in normal aged human brain [9].

Transcript expression of the immediate-early gene arc, activity-regulated cytoskeleton-associated protein, is strongly increased in cerebral cortex following exposure to a novel enriched environment or during learning [17]. This increase in expression is attenuated in animals injured by entorhinal cortex lesions. Our comparison of mouse and human transcript expression showed that basal arc expression dramatically decreases with age in both mouse and human. The possibility that age-associated depression of basal levels of arc may affect the ability of aged animals to learn or to respond adequately to novel environments merits further study.

In an earlier study we compared the effects of aging in two mouse strains (CB6F1 and B6C3F1), using two separate types of Affymetrix gene chips (U74av2 and MG430). Despite these differences the expression correlation ($R = 0.96$) was much higher than in the current study [1]. Many potential confounders could reduce the significance of the correlation found between mouse and human brain tissues. This includes the fact that in the human cases, a significant time (mean of 17 h) elapsed between death and brain mRNA preparation and this may have allowed significant degradation to occur. Furthermore, the human population was heterogeneous—71% Caucasian, 18% Hispanic, 8% African American and 2% Asian; 30 males and 9 females. The mouse and human brain regions utilized were somewhat different. The human study used the BA9 and BA47 cortical regions, while we used cortex plus hippocampus. Added to these sources of variability is the experimental error inherent in measurement of transcript levels using microarrays. To mitigate this, both mouse and human microarray expression levels were confirmed by RT-PCR, extensively in the case of the human data. Despite these procedural differences, it is remarkable that a highly significant correlation was derived.

The overall parallel changes in expression of certain classes of gene found to occur during senescence serves to validate the use of the mouse in order to model certain aspects of human aging.

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