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Brief communication

Reciprocal phylogenetic conservation of molecular aging in mouse and human brain

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Abstract

Studies of age-related molecular profiles have separately focused on the human and rodent brains, but the extent to which each organism predicts molecular events across species for the global signature of aging and for specific biological functions has only begun to be characterized. We previously showed that the molecular correlates of aging in the mouse cortex moderately, but significantly, predicted transcript changes in human frontal cortex. Using orthologous gene links between large-scale gene expression datasets, we now report a similar reciprocal human-to-mouse prediction of molecular aging in frontal cortex, but a limited and variable conservation of age-effects across a wide spectrum of biological functions. Thus, the moderate transcriptome correlations and partial functional concordance between late-life human and rodent cohorts (13–77 years in humans and 3–24 months in mice) suggest limitations of the mouse to model normal aging of the human brain cortex.

Keywords: Molecular aging; Human; Mouse; Microarray; Frontal cortex; Ortholog changes; Directional correlation

1. Introduction

As a universal feature of living organisms, many molecular components of aging are shared across phyla, including humans (McCarroll et al., 2004). In mammalian systems, aging is also associated with brain-related impairments including cognitive and motor functions and is a major risk factor for neurodegenerative and psychiatric disorders (Yankner et al., 2008). While critical biological features such as increased oxidative stress, disturbances in energy metabolism and inflammation-like processes influence brain aging (Weindruch and Prolla, 2002), the complexity and terminally differentiated nature of the mammalian brain are associated with unique morphological, cellular and molecular features, which are subjected to additional mechanisms of age-related changes (Yankner et al., 2008). Moreover, while the mouse is the preferred model for genetic studies of mechanisms that are relevant to the human brain, considerable evolutionary differences exist in brain structure. Finally, as mortality mostly results from catastrophic system failures in peripheral organs, it is not clear whether differences in lifespan allow for mouse brain aging to recapitulate the extent of changes occurring during aging of the human brain. To date, only a few studies have investigated normal aging of the human brain (Lu et al., 2004; Erraji-BenChekroun et al., 2005; Berchtold et al., 2008). Comparative analyses have reported considerable species differences in affected gene categories and concluded that only a relatively small subset of genes displayed conserved changes in transcript levels with age, although these studies were restricted to single gene approaches (Sharman et al., 2005), or to cohorts of very-old subjects (27–106 years old) (Lu et al., 2004; Loerch et al., 2008). Here, using measures of directional correlations to assess the extent to which each species predicts changes in the other species, combined with large-scale functional analyses, we provide additional evidence in support of a statistically robust conservation of overall age-effects during normal aging (humans, 13–79 years and mice, 3–24 months),

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but of variable scope across a wide spectrum of biological functions.

2. Methods

2.1. Human cohort

Postmortem samples from prefrontal cortex Brodmann areas 9 and 47 (BA9–BA47) in 39 subjects (13–79 age range) free of neurodegenerative disease were processed on Affymetrix U133A arrays (Affymetrix Inc., Santa Clara, CA). Cohort, sample dissections, microarray hybridization and the analysis of the molecular correlates of aging have been previously described (Erraji-BenChekroun et al., 2005). Due to the continuous distribution of age range, Pearson correlation coefficients (age versus gene transcript levels for all 39 subjects) were used to select genes ($p < 0.05$; p-value of Pearson correlation calculated from conventional t-test). Pearson correlation is more adapted and powerful to detect effects when applied to the continuous age range of the human cohort, while the categorical distribution of the mouse samples (see next) can only be assessed by ANOVA. This was confirmed in that dividing the human data into categorical sets (0–19, 20–39, 40–59, and ≥60 years old) and performing an ANOVA yielded consistent but fewer results than with Pearson correlation. Age fold changes [$\log_2$ −(>65 years; $n = 8$ for BA9 and $n = 11$ for BA47)/(<35 years; $n = 7$ for BA9 and $n = 8$ for BA47)] were also calculated.

2.2. Mouse data set

3-, 10-, 18- and 24-month-old 129SvEv wild-type mice were used. The cohort, breeding conditions, cortical sampling, microarray hybridization (Affymetrix MOE 430-2.0 arrays) and the analysis of the molecular correlates of aging have been previously described (Sibille et al., 2007). Due to the categorical distribution of ages, the effect of age could only be assessed by the analysis of variance ($p < 0.05$; $n = 3$–4 per age-group) and age fold changes [$\log_2$ −(24 months/3–10 months)]. For both the human and mouse, we used p-value threshold 0.05 without considering multiple comparisons, due to weak signal and small sample size in both studies.

2.3. Orthologous microarray gene probeset mapping

Orthologous microarray gene probeset mapping was carried out between the human (HG-U133A_2; 22283 probesets) and rodent (MOE 430_2; 45101 probesets) arrays using the Netaffx webtool (Affymetrix Inc., Santa Clara, CA). To reduce the redundancy of array probesets targeting the same genes and to enrich our database in age-effects, the probeset with the highest correlation with age in the human data or the probeset with the smallest ANOVA p-value in the mouse data was kept when multiple probesets map to the same gene. This procedure left 865 unique genes for further analysis.

2.4. Directional correlation coefficient

$r(\mathrm{D1} \Rightarrow \mathrm{D2})$ was calculated in cross-species datasets D1 and D2. In contrast to traditional Pearson correlation, the directional correlation measures the fitness of significant genes in D1 to predict D2, and conversely of D2 to predict D1. For instance, the statistically significant genes in D1 (named G_1) were first selected, and the Pearson correlation of the mean log-ratios (see Sections 2.1 and 2.2) of D1 and D2 in this restricted D1-significant gene set (G_1) was calculated. Although the dataset distributions dictated different statistical criteria for age-effects in human (Pearson correlation) and mice (ANOVA) (see Sections 2.1 and 2.2), the use of log-ratios of age-effects ensured direct comparisons across species. Thus, directional correlations are not symmetric [$r(\mathrm{D1} \Rightarrow \mathrm{D2}) \neq r(\mathrm{D2} \Rightarrow \mathrm{D1})$] (Table 1). The rigorous mathematical formulation of "directional correlation coefficient" is provided in the supplements. The statistical significance of directional correlation coefficients was assessed by permutation tests. For instance, for $r(\mathrm{D1} \Rightarrow \mathrm{D2})$, a randomly selected D1 gene set of the same size was selected from age-irrelevant genes (i.e. genes not selected by p-value or fold-change criteria) and a directional correlation was similarly calculated. The procedure was repeated 100,000 times to generate a null distribution against which p-values could be assessed. To further assess the confidence interval of the observed directional correlations between mouse and human data sets, bootstrapped data sets of BA9, BA47 and mouse were generated and the directional correlations were calculated to derive the 95% confidence intervals (repeat B = 100 times).

2.5. Parametric analysis of gene set enrichment (PAGE)

To investigate the cumulative effect of aging on functionally related genes (i.e. gene ontology, biological pathways, etc.), we applied a parametric statistical analysis model (PAGE) (Kim and Volsky, 2005) of the original gene set enrichment analysis (Subramanian et al., 2007), as it has been shown to result in larger numbers of significantly over-presented gene sets. Here, the functional analyses were applied to the sets of all detected transcripts (∼50% of probesets) in the respective species. Gene sets with less than 10 genes were not retained. Z-scores and associated p-values were used. Detailed methods are provided in the supplements.

3. Results

3.1. Human–mouse reciprocal predictions of age-related gene expression changes

We investigated the degree to which gene expression correlates of aging predicted similar changes between the human prefrontal cortex (two areas) and the mouse frontal cortex. In short, gene transcript changes were identified in one species (e.g., human cortex), and the correlation of changes for

Table 1
Mouse–human directional correlations of age-effects. *R*, correlation factor; *p*, *p*-value of permutation test for individual directional correlation (see Section 2). Confidence intervals (C.I.) were derived from bootstrap analyses. The non-significant *p*-value for the BA47 to mouse directional correlation using correlation criteria only may reflect a lower power due to fewer selected genes, as a greater and significant directional correlation was observed when the extent of age-related changes was applied as the initial selection criterion.

Criterion for cut-off (number of genes selected)		Mouse FC to human BA9	Mouse FC to human BA47
ANOVA $p < 0.05$	*R* (*p*)	0.21 (0.0296)	0.32 (0.0015)
475 genes	C.I.	[0.16–0.25]	[0.28–0.36]
abs(alr) > 0.26	*R* (*p*)	0.35 (0.0101)	0.47 (0.0007)
139 genes	C.I.	[0.31–0.39]	[0.43–0.51]
Criterion for cut-off (number of genes selected)		**Human BA9 to mouse FC**	**Human BA47 to mouse FC**
Correlation $p < 0.05$	*R* (*p*)	0.24 (0.0402)	0.18 (0.5477)
BA9/47: 189/119 genes	C.I.	[0.19–0.29]	[0.12–0.24]
abs(alr) > 0.26	*R* (*p*)	0.31 (0.0003)	0.31 (0.0129)
BA9/47: 108/135 genes	C.I.	[0.26–0.36]	[0.28–0.34]

these genes was calculated for ortholog gene changes in the aging mouse cortex. Consistent directional correlations were identified between groups of orthologous genes from mouse-to-human, and conversely, from human-to-mouse (Table 1), although fold-change criteria [changes greater than 20%] yielded more robust findings than statistical criteria only [i.e., $p(\text{age } R) < 0.05$], as demonstrated by permutation of the results (Table 1; see Section 2). The technical reliability of the two datasets was previously demonstrated by quantitative real-time PCR (array-qPCR correlations >0.85 in both datasets; Erraji-BenChekroun et al., 2005; Sibille et al., 2007).

3.2. Human–mouse conservation of age-effects on biological functions

The cumulative effect of age-related changes in gene transcript levels for sets of genes participating in common biological functions revealed 200 gene groups affected by age in humans, and 62 in mice. Note that all expressed transcripts in the respective species were used for these analyses and weighted according to their age-effects (see Section 2). This means that biological functions affected through different, but complementary, sets of genes across species, would be identified by this approach. Biological functions identified in BA9 and BA47 were highly similar (*Z*-scores $R = 0.92$), so BA47 results only were compared to the mouse results. Accordingly, results were compared for 246 gene groups with significant effects in at least one species and with a minimum of 10 included genes in both species (Table S1) and regrouped according to main biological functions (Table 2). Results indicate that age-related changes were moderately concordant for immune function/inflammation, receptor activity/signaling and translation (i.e. few similar gene subgroups affected in both species; Table 2 and Table S1) and opposite for development/cell cycle and DNA replication/transcription. Ca-dependent functions did not reach group age-effects in mice. Other main biological functions displayed non-conclusive results.

4. Discussion

We previously reported that aging in the mouse cortex predicted equivalent changes in the human cortex (Sibille et al., 2007). Here, applying a systematic comparison and calculating measures of directional correlations between human and mouse brain transcriptomes, we report reciprocal and similar predictions of age-related gene transcript changes between the human and mouse brain cortices (Table 1). These effects were confirmed across two different areas of the human prefrontal cortex. In contrast to traditional Pearson correlation, the directional correlation measures the fitness of significant genes in one system to predict changes in the other system. Accordingly, the present results demonstrate that the higher complexity of the human cortex—implicitly providing additional and distinct substrates for age-related effects—and the large difference in aging timeframes between human and mice, did not result in lower overall prediction of age-related molecular changes in the human-to-mouse direction. We also note the slightly higher directional correlation between human BA47 (i.e. orbital ventral prefrontal cortex) and mouse frontal cortex, compared to BA9 (i.e. dorsolateral prefrontal cortex), although the underlying reasons are not known.

However, phylogenetic similarities and differences were revealed in terms of affected biological functions. Conserved changes relating to immune function/inflammation are consistent with previous reports (see review in Yankner et al., 2008). The observed concordance in direction, but large discrepancy in scope, in downregulated receptor function and signaling (~40% of age-affected gene groups in humans versus ~21% in mice; Table 2) is also consistent with the relative paucity of neuron-related changes in the mouse brain compared to the large and robust downregulation observed in aging humans (Lu et al., 2004; Erraji-BenChekroun et al., 2005; Loerch et al., 2008). Opposite or potentially unaffected age-related correlates for DNA replication/transcription, metabolism and cellular structure may represent either a lack of full age-effect in 24-month-old mice, or more fundamental differences in age-related mechanisms. For instance, opposite

Table 2
Summary of the parallel functional analysis of age-related transcriptome changes in human and mouse cortex. Values presented are from human BA47, as this region demonstrated higher correlations with mouse frontal cortex, although results were similar for BA9. "number of groups" indicate significant gene groups regrouped under main biological functions; (% totals) indicate the proportion of the total number of gene groups in that category. Z-scores summarize the overall direction of changes within gene groups. "Z-score signs" indicate the proportion of up- and downregulated gene groups per biological function.

Cellular/biological function	Human			Mouse			Mouse/human
	Main age-effect	Number of groups (% total)	Z-scores signs	Main age-effect	Number of groups	Z-scores signs	In common (% concordant)
Concordant							
Immune function/inflammation	Increase	2 (1%)	2+	Increase	3 (4.8%)	3+	1 (100%)
Metabolism	Increase/decrease	20 (10%)	11+/9−	Increase	7 (11.3%)	6+/1−	2 (100%)
Receptor activity/signaling	Decrease	79 (39.5%)	78−/1+	Decrease	13 (21%)	4+/9−	4 (100%)
Translation	Increase	18 (9%)	18+	Increase	9 (14.5%)	8+/1−	8 (100%)
Opposite							
Development/cell cycle[a]	Increase	9 (4.5%)	8+/1−	Decrease	13 (21%)	13−	1 (0%)
DNA replication/transcription	Increase	26 (13%)	26+	Decrease	14 (22.6%)	14−	1 (0%)
Others							
Ca-dependent function	Decrease	12 (6%)	1+/11−	na	0	0	0 (0%)
Cellular structure	Decrease	26 (13%)	8+/18−	Increase/decrease	3 (4.8%)	2+/1−	0 (0%)
Miscellaneous		8 (4%)	6+/2−		0		
Total GO groups		200			62		

[a] Increased apoptosis-related GO in human and decreased neurogenesis-related GO in mouse (details in Table S1).

changes between the mouse and human brain in cell cycle and development-related gene groups may result in similar cellular phenotypes through increased apoptosis-related functions in human and decreased neurogenesis-related functions in mouse (Table S1).

Together, the major implication of these findings is that the mouse may at best represent a moderately adequate model for normal aging of the human brain, depending on the system and biological function investigated (Table 2). It is conceivable that these results may reflect the difference in time for somatic mutations and oxidative damage to accumulate in the rodent brain, resulting for instance in greater demand for DNA repair in humans compared to the mouse brain (Lu et al., 2004), although this was not assessed at the functional level. Alternatively, contributing causes to the observed differences may reside in adaptive differences in age-related genetic programs between humans and mice. A more accurate comparison for human brain aging may be with older mice (i.e. 30–36 months of age), although Loerch et al. (2008) suggest that this is not the case at the molecular level. Moreover, depending on the strain, older mice commonly used for genetic manipulations develop either debilitating skin lesions (C57B6 strain), or severe locomotor deficits (129SvJ and C57B6) (Crawley, 2000), which considerably limit their use for behavioral investigations that are relevant to cognitive or emotion-related brain functions, notwithstanding the logistical difficulties of maintaining such colonies.

In summary, these results provide a robust and independent confirmation of previous findings obtained in very-old human subjects and mice (Jiang et al., 2001; Loerch et al., 2008), extend the cross-species characterization of age-related changes to normal aging in both species (65–77 years in humans and 24 months in mice) and to a different mouse strain (129SvJ here; C57B6 in Jiang et al., 2001) and together present a concise overview of the extent of the mouse–human phylogenetic conservation of the impact of normal aging in brain cortex. Although the two species are very close at the cellular and molecular level, the major discrepancy in the extent (Sharman et al., 2005; Loerch et al., 2008) and relative concordance (Table 2) of cellular signaling-related changes occurring with increasing age, strongly suggest that aging of the mouse cortex does not recapitulate the molecular intricacies underlying the functional compensations and deficits that may be mostly responsible for cognitive decline and affective changes in human aging. It remains to be seen if these observations extend to subcortical brain regions, especially in areas relating to neuroendocrine and mood regulation, two functions known to be affected during normal human aging (Lerer et al., 1999).

Conflict of interest

The authors hereby declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2009.08.004.

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