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DNA Microarray Analysis of the Aging Brain

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Abstract

To examine molecular events associated with brain aging and its retardation by caloric restriction (CR), we have employed high-density oligonucleotide arrays providing data on 6347 genes to define transcriptional patterns in two brain regions (cerebellum and neocortex). Male C57BL/6 mice were either fed normally or subjected to CR. To investigate aging, 5 month (young adult) and 30 month-old normally fed mice were compared. To study CR, 30 month-old control and CR mice were compared. In both brain regions, aging resulted in a gene expression profile suggestive of a marked inflammatory response, oxidative stress and reduced neuronal plasticity and neurotrophic support. In the brain, CR selectively attenuated the age-associated induction of genes encoding inflammatory and stress responses. In addition to providing an improved understanding of the aging process, the use of DNA microarrays generates panels of hundreds of transcriptional biomarkers of molecular aging, providing a new tool to measure biological age on a tissue-specific basis. These studies suggest that genomic approaches may be useful in understanding the molecular basis of the aging process in experimental animals.

Introduction

Several studies have identified age-associated alterations in olfaction in humans, including increased odor detection threshold (Deems and Doty, 1987; Stevens and Spencer, 1994), decreased odor identification (Murphy *et al*., 1997) and decreased odor discrimination (Hulshof Pol *et al*., 2000). Chemosensory decrements in the elderly may contribute to poor nutrition, food poisoning and overexposure to environmentally hazardous chemicals (Schiffman, 1997). The molecular basis of age-related chemosensory decrements and how they relate to cognitive and motor impairments remains unclear. In order to gain a better understanding of general aspects of brain aging, we are currently using DNA microarray analysis to study caloric restriction (CR) as a model system of aging retardation in mammals (Lee *et al*., 1999, 2000; Kayo *et al.*, 2001). These studies have shown the feasibility and utility of gene expression profiling to identify basic aspects of brain aging. The technology is readily applicable to specific areas of aging investigation, including studies of the molecular basis of age-related chemosensory alterations in experimental animals.

CR retards the aging process

CR retards the aging process in laboratory rodents as characterized by a delayed occurrence or complete prevention of a broad spectrum of age-associated pathophysiological changes and a 30–50% increase in maximum lifespan (Weindruch and Walford, 1998). The maximum lifespan of fish, rotifers, spiders and other non-mammals is also extended by CR (Weindruch and Walford, 1998). An active area of research in biological gerontology concerns the mechanisms by which CR retards the aging process. There are five classes of interrelated and non-exclusive explanations for CR's mechanism: (i) decreases in oxidative stress (Sohal and Weindruch, 1996); (ii) decreases in glycation or glycoxidation (Kristal and Yu, 1992); (iii) decreases in body temperature and circulating thyroid hormone levels associated with a hypometabolic state (Walford and Spindler, 1997); (iv) alterations in gene expression and protein degradation (Van Remmen *et al*., 1995); and (v) neuroendocrine changes (Nelson *et al*., 1995).

Oligonucleotide microarrays as a tool to evaluate aging interventions

DNA microarrays are likely to revolutionize biomedical research through the simultaneous analysis of gene expression patterns of whole genomes (Lander, 1999). Large-scale analysis of transcriptional responses has proven to be useful in the analysis of complex biological phenomena such as metabolism in *Saccharomyces cerevisiae* (DeRisi *et al*., 1997) and tumorigenesis in humans (Perou *et al*., 1999). Since such arrays are synthesized based on sequence information, they provide a direct link between differential gene expression

patterns and information readily available in public genomic databases. We have used Affymetrix oligonucleotide arrays containing tens of thousands of gene-specific oligonucleotides synthesized *in situ* using light-directed, solid-phase combinatorial chemistry (Lipshutz *et al.*, 1999). The Affymetrix arrays used initially represented 6347 genes and contained 40 specific oligonucleotide probes, which are 25 bp long, for each gene represented in the array. Twenty of these oligonucleotides represent perfect matches (PM probes) to the gene of interest along its length. Another 20 oligonucleotides contain a single mismatch (MM probes) as compared with the PM probes. In order to calculate an average signal intensity, hybridization intensity from each MM probe is subtracted from that of its PM counterpart and the values are averaged for the 20 pairs.

In a typical experiment, mRNA populations from individual animals are used to generate cRNA pools (see Materials and methods), which are then hybridized to individual oligonucleotide arrays. Because samples are applied to different microarrays and the data from such microarrays are compared, normalization between arrays is required. Such normalization can be performed by using the hybridization intensity of specific genes as internal controls, in the same manner that one uses the hybridization of housekeeping genes such as GAPDH or β-actin in Northern blots. Alternatively, the total hybridization intensity of DNA microarrays can be used as normalization factors. Figure 1 demonstrates the image of two such arrays and the fluorescence patterns of two genes, derived from the comparison of heart samples from young (5 month) and old (30 month) C57BL6/J mice (unpublished results).

We are currently characterizing the gene expression profile associated with the aging process in several organs of mice, including skeletal muscle, cerebellum, hippocampus,

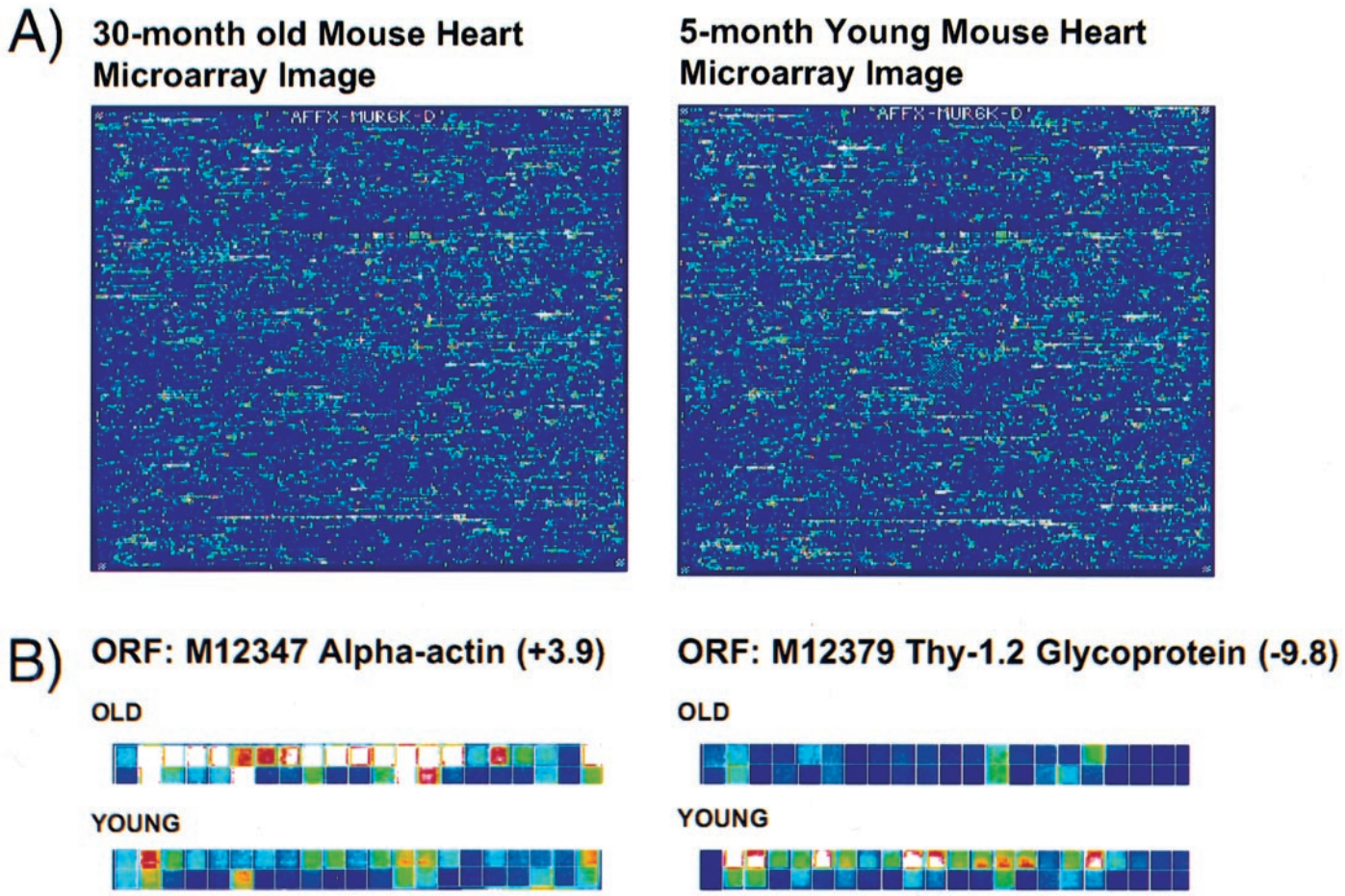

Figure 1 DNA microarray analysis of aging. **(A)** Overall hybridization patterns of 6347 genes in the heart of a young adult (5 month) and an aged (30 month) mouse. Note that experiments with Affymetrix arrays involve separate hybridizations to DNA microarrays for each condition (in this case age) under study. DNA microarrays are not re-usable, so different hybridizations are performed on different DNA microarrays. **(B)** Two specific probe families contained in the DNA microarray, those representing the gene α-actin and Thy-1.2 Glycoprotein. α-actin increases in expression with aging by 3.9-fold, whereas Thy-1.2 Glycoprotein decreases in expression. Note that the top row of probe features shows strongest hybridization (perfect match probes), whereas the lower row displays lower hybridization intensity (mismatched probes). Blue represents no hybridization, white represents the strongest hybridization.

neocortex, kidney, liver, heart and lymphocytes. Although this review emphasizes results that we have obtained in the brain using DNA microarrays that contain ~6500 genes, we are currently using third generation Affymetrix murine genome U74 DNA chips that can obtain gene expression information on >30 000 cDNAs and ESTs.

Materials and methods

Animals and diets

Details on the methods employed to house and feed male C57BL/6 mice, a commonly used model in aging research with an average lifespan of ~30 months, were recently described (Pugh *et al*., 1999). Briefly, mice were purchased from Charles River Laboratories (Wilmington, MA) at 1.5 months of age. After receipt in Madison, the mice were housed singly in the specific pathogen-free Shared Aging Rodent Facility at the Madison Veterans Administration Geriatric Research, Education and Clinical Center, and provided a nonpurified diet (PLI 5001; Purina Labs, St Louis, MO) and acidified water *ad libitum* for 1 week. The mice were then allocated into two groups and fed one of two nearly isocaloric (~4.1 kcal/g), semipurified diets. Each mouse in the control group was fed 84 kcal/week of the control diet (TD91349; Teklad, Madison, WI) which is ~5–20% less than the range of individual *ad libitum* intakes. This dietary intake was used so that the control mice were not obese and retained motor activity up to the age of sacrifice. Each mouse subjected to CR was fed 62 kcal/week of the restricted diet (TD91351; Teklad), resulting in a 26% reduction of caloric intake. The latter diet was enriched in protein, vitamins and minerals such that CR and control mice were fed nearly identical amounts of these components. The fat component, corn oil, was at the same level (13.5%) in both diets, leading to a 26% reduction in fat intake for the CR mice. The adult body weights of the mice averaged ~32 g for controls and ~23 g for those on CR. Mice were killed by rapid cervical dislocation, autopsied to exclude animals showing overt disease and several tissues collected and immediately flash-frozen in liquid nitrogen and stored at –80°C. All aspects of animal care were approved by the appropriate committees and conformed with institutional guidelines.

Sample preparations and hybridizations

Total RNA was extracted from frozen tissue using TRIZOL reagent (Life Technologies) and a power homogenizer (Fisher Scientific) with the addition of chloroform for the phase separation before isopropyl alcohol precipitation of total RNA. Poly(A)$^+$ RNA was purified from the total RNA with oligo-dT-linked Oligotex resin (Qiagen). One microgram of poly(A)$^+$ RNA was converted into double-stranded cDNA (ds-cDNA) using SuperScript Choice System (Life Technologies) with an oligo dT primer containing a T7 RNA polymerase promoter region (Genset). After second strand synthesis, the reaction mixture was extracted with phenol/chloroform/isoamyl alcohol. Phase Lock Gel (5 Prime → 3 Prime Inc.) was used to increase ds-cDNA recovery. The ds-cDNA was collected by ethanol precipitation. The pellet was resuspended in 3 µl of DEPC-treated water. *In vitro* transcription was performed using a T7 Megascript Kit (Ambion) with 1.5 µl of ds-cDNA template in the presence of a mixture of unlabeled ATP, CTP, GTP and UTP, and biotin-labeled CTP and UTP [bio-11-CTP and bio-16-UTP (Enzo)]. Biotin-labeled cRNA was purified using a RNeasy affinity column (Qiagen). The amount of biotin-labeled cRNA was determined by measuring absorbance at 260 nm. Biotin-labeled cRNA was fragmented randomly to sizes ranging from 35 to 200 bases by incubating at 94°C for 35 min in 40 mM Tris-acetate, pH 8.1, 100 mM potassium acetate and 30 mM magnesium acetate. The hybridization solutions contained 100 mM MES, 1 M [Na^+], 20 mM EDTA and 0.01% Tween 20. In addition, the hybridization solutions contained 50 pM oligonucleotide B2 (a biotin-labeled control oligonucleotide used for making grid alignments), 0.1 mg/ml herring sperm DNA and 0.5 mg/ml acetylated BSA. The final concentration of fragmented cRNA was 0.05 g/l in the hybridization solutions. Hybridization solutions were heated to 99°C for 5 min followed by 45°C for 5 min prior to placing in the gene chips. cRNA (10 µg) was placed in the gene chip. Hybridizations were carried out at 45°C for 16 h with mixing on a rotisserie at 60 r.p.m. Following hybridization, the hybridization solutions were removed and the gene chips were installed in a fluidics system for washes and staining. The fluidics system (Affymetrix GeneChip Fluidics Station 400) performed two post-hybridization washes (a non-stringent wash and a stringent wash), staining with streptavidin–phycoerythrin and one post-stain wash. The gene chips were read at a resolution of 6 µm using a Hewlett Packard GeneArray Scanner. Data collected from two scanned images were used for the analysis.

Data collection

All calculations were performed by Affymetrix software. To determine the quantitative RNA abundance, the average of the differences representing PM minus MM for each gene-specific probe family was calculated, after discarding the maximum, the minimum and any outliers beyond three standard deviations. For example, to calculate fold changes (*FC*) between data sets obtained from young (y) versus old (o) mice, the following formula was used:

$$FC = (SI_o - SI_y)/(\text{the smallest of either } SI_y \text{ or } Si_o) + 1 \text{ if } SI_o = SI_o \text{ or } -1 \text{ if } SI_o < SI_y$$

where SI_o is the average signal intensity from a gene-specific probe family from an old mouse and SI_y is that from a young mouse.

Alternatively, if the Q_{factor}, a measure of the non-specific

fluorescence intensity background, was larger than the smallest of either SI_y or SI_o, the *FC* was calculated as:

$$FC = (SI_o - SI_y)/Q_{factor}$$

The Q_{factor} is automatically calculated for different regions of the microarray and therefore minimizes the calculation of spurious fold changes by replacing very low average signal intensities in the *FC* equation. Average of pairwise comparisons were made between study groups, using Excel software. As an example, in our studies each 5-month-old mouse was compared with each 30-month-old mouse generating a total of nine pairwise comparisons. Correlation coefficients between animals of the same experimental group were >0.98 in all cases, indicating very small variability between animals.

Statistical Analysis

A key challenge to the development of statistical methods for microarray data is that sample size (i.e. the number of animals, tissues or independent replicas in tissue culture experiments) is often small, but the number of measurements is very large. Additionally, the expression levels for individual genes may not be independent. Fold changes, which are typically used to report microarray data, are measures of magnitude and not direct measures of strength of evidence for an effect. When comparing large numbers of genes between two specific age groups, it is likely that a number of genes will differ in expression by 2-fold or more (the usual cutoff in DNA microarray experiments) between the experimental groups by chance (type 1 errors). In other words, such changes would not be observed if a different set of individuals from the two age groups was compared. The observed number of false positives depends on the individual variance of genes, because genes that have large variations in expression levels are more likely to result in type 1 errors. It has been calculated (Miller *et al*., 2001) that in a study design containing three young versus three old mice (such as we employed) and an average gene coefficient of variance (*CV*) of 30, 58 false positives would be generated by chance if the expression of 10 000 genes is analyzed. We have estimated that if the number of genes to be screened is 6347 (as contained in the MU6500 DNA chip) the per gene level required is ~0.0000081 to keep the overall experiment-wide type 1 error to be 0.05, if one uses conventional statistic methodology. In order to have ~99% power to detect a difference of 0.2 standard deviations, 2400 samples (mice in our case) would have to be sampled for microarray testing. Clearly, novel statistical methods for the analysis of DNA microarray data are the most urgent need in the field.

Our own study design of the gene expression alterations in the aging brain (Lee *et al*., 2000) involved a first step of data filtering that removed most genes with high *CV*s, since we only reported genes that had a fold change >1.3 + the SEM. After removal of genes that did not fit these criteria, the average *CV* for all remaining genes (a few hundred) was only 20.9. According to Miller's simulation of 10 000 genes with this particular average *CV* (Miller *et al*., 2001), one would expect to observe only six 'false positive genes' displaying a >2× alteration in expression and no such genes displaying a >3-fold alteration in gene expression. We observed >12 genes displaying a 3-fold or greater alteration in gene expression in the cerebellum of mice, indicating that the majority of age-related alterations in gene expression are unlikely to be type 1 errors.

A 'two-step approach' to reduce type 1 errors has also proposed (Miller *et al*., 2001). This involves an initial, exploratory selection of genes that satisfy a given statistical criteria (using an initial level), followed by 'confirmation testing' of these genes with an independent group of animals (using a second level). However, it is unclear if this method indeed helps investigators to make fewer type 1 (false positive) and type 2 (false negative) errors. We have argued that the two-stage procedure offered by Miller *et al* is unnecessary to hold the overall experiment-wise type 1 error rate to some desired level (Prolla *et al*., 2001). Based on examination of many microarray data sets, we feel that it is likely that (i) many genes have dependent (correlated) expression levels (i.e. are co-expressed) and (ii) with profound independent variables such as aging, the null hypothesis of no group differences in expression is false for many genes, but the relative difference in expression across the two groups may be modest (e.g. 30–100%). Therefore, we are developing a sequence of procedures that involve finite mixture modeling and bootstrap inference in order to address significance testing for microarray data (Allison *et al.*, 2001). This set of procedures is based on the idea that when many statistical tests are conducted, one obtains a distribution of test statistics and corresponding *P*-values and that there is information available in this distribution that can be exploited. In particular, the method employs the observation that under the alternative, more likely hypothesis (i. e. that there is in fact a difference between groups) the distribution of *P*-values for individual genes will not be uniform.

Our initial analysis involving finite mixture modeling and bootstrap inference of our data collected in aging skeletal muscle in mice (three mice/age group, 6347 genes screened) indicates that even among genes for which there is a real difference in expression, we only expect *P*-values to be 0.17 on average if three animals/group are used in the analysis (Allison *et al*, 2001). We have also determined that as long as the *P*-value for an individual gene is <0.35, there is a >50% chance that the gene identified is a gene for which there is a real difference in expression. These results underlie the fact that, given the small sample size in most microarray studies, power is low and that conventional significance testing with an level of 0.05 or smaller would lead to many false negatives (i.e. misses). Therefore, false negatives, as opposed

to false positives, are likely to represent the major statistical issue in DNA microarray analysis.

Results and discussion

The gene expression profile of aging in the mouse brain

Of the 6347 genes surveyed, only 67 (1%) displayed an increase >1.7-fold in expression levels with aging in the neocortex, whereas 63 (1%) displayed an increase of >2.1-fold expression with aging in the cerebellum (see www.wisc.edu/genetics/CATG/prolla/data/aging/index.html for a full listing of genes). A subset of these genes was used for a comparison between oligonucleotide microarray data and TaqMan real-time quantitative PCR assays, which demonstrated excellent overall agreement between the methods (Lee *et al*., 2000). Of the upregulated genes, 20% (14/67) and 27% (17/63) could be assigned to an immune or inflammatory response in the neocortex and cerebellum, respectively (Table 1). Transcriptional alterations of several genes in this category were shared by the two brain regions, although fold changes tended to be higher in the cerebellum. Interestingly, we saw a concerted induction of the complement cascade components C4, C1qA, C1qB and C1qC. Complement proteins are found in the senile plaques characteristic of Alzheimer's disease (Akiyama *et al*., 2000).

Similar to previous data obtained from the gastrocnemius muscle (Lee *et al*., 1999), genes involved in a stress response (consistent with a state of oxidative stress and accumulation of damaged proteins) accounted for 24% (16/67) and 13% (8/63) of genes highly induced in the neocortex and cerebellum, respectively, of old animals (Table 2). These included the four cathepsins (lysosomal proteases) and the several heat-shock factors (e.g. Hsp40, Hsp27, Hsp59). Also induced with age was the gene encoding ubiquitin, a major stress-inducible transcript that marks altered proteins for degradation by the ubiquitin/ATP-dependent proteosome pathway. The accumulation of intracellular inclusions containing misfolded, ubiquitinated proteins is a hallmark of several neurodegenerative disorders (Koshy *et al*., 1997; Arnold *et al*., 1998). Possibly, the molecular basis for the induction of chaperones and lysosomal proteases in the aged brain is protein oxidation subsequent to inflammation and mitochondrial ROS production.

We also observed an induction of the early response genes junB and c-fos, which are co-induced in response to neocortical injury or hypoxic stress and GFAP, a marker of reactive astrocytosis induced in the aging brain (Table 1). Taken as a whole, our observations support the concept that the aging process in the brain is associated with a state of heightened immune reactivity and oxidative stress accompanied by the accumulation of altered or misfolded proteins. Therefore, a chronic state of oxidative stress appears to be a common transcriptional feature of aging in both brain and skeletal muscle.

Several transcripts which declined in expression encode nuclear genes involved in mitochondrial functions. These include NADP transhydrogenase, cytochrome-C-oxidase subunit VIII and the ATP synthetase-gamma and delta chains which are all either components of the mitochondrial electron transport system or support its function. This transcriptional profile suggests that mitochondrial function may be compromised in the aged brain, parallel to observations in skeletal muscle (Lee *et al*., 1999).

Table 1 Transcriptional evidence for an inflammatory response with aging in the mouse brain

Fold increase ↑ (SE)			CR prevention (%)	
Neocortex	Cerebellum	Gene	N	C
4.9 (0.2)	3.7 (0.3)	complement C4	52	0
2.2 (0.2)	6.4 (0.9)	lysozyme C	64	88
2.0 (0.2)	ND	CD40l receptor	58	–
1.9 (0.4)	4.7 (1.0)	cyclophilin C-AP	100	0
1.9 (0.2)	3.9 (0.1)	Mps1	0	56
1.8 (0.1)	ND	C1q-B chain	100	–
1.8 (0.1)	4.1 (0.6)	C1q-C chain	75	56
1.7 (0.1)	4.4 (0.2)	C1q-A chain	100	80
1.7 (0.1)	ND	Cox-1	0	–

ND = not different with age.

Table 2 Transcriptional evidence for a stress response with aging in the mouse brain

Fold increase ↑ (SE)			CR prevention (%)	
Neocortex	Cerebellum	Gene	N	C
2.3 (0.3)	3.8 (0.7)	GFAP	59	61
1.7 (0.3)	5.6 (2.6)	c-fos	100	59
1.7 (0.1)	4.7 (0.3)	cathepsin S	56	62
1.8 (0.1)	2.6 (0.2)	cathepsin D	64	38
1.9 (0.1)	2.1 (0.2)	cathepsin Z	70	64
ND	2.9 (0.3)	cathepsin H	–	55
1.8 (0.2)	3.1 (0.4)	HSP40 homolog 1	51	100
1.7 (0.2)	ND	HSP40 homolog 2	0	–
1.8 (0.3)	ND	Gadd153	0	–

ND = not different with age.

CR is associated with a transcriptional reprogramming in the brain

Of the largest age-associated changes in the neocortex (1.7-fold or more), 30% (34/114) were prevented either completely or partially by CR. Remarkably, the effect of CR on age-associated alterations in gene expression was highly dependent on transcript class: CR influenced only 20% (2/10) of the observed decreases in expression of genes involved in neuronal growth and plasticity, whereas it

prevented 50% (8/16) of the induction of stress response and 65% (11/17) of immune-response-related genes. A similar pattern was observed in the cerebellum. The effects of CR on immune- and stress-related transcripts agree with studies suggesting that reductions in both autoimmunity (Weindruch and Walford, 1998) and oxidative damage (Sohal *et al*., 1994) occur in the brain of CR mice.

As was observed in muscle, CR shifted the activity of several genes that were unaltered in expression with age (see www.wisc.edu/genetics/CATG/prolla/data/aging/index.html for a full listing of genes affected by CR in the neocortex). As compared with age-matched controls, CR induced the expression of 120 genes by 1.7-fold or more in the neocortex, which represent 1.9% of the genes surveyed (Table 3). One of the largest classes of transcripts induced by CR was comprised of growth and neurotrophic factors (9%). This class included the developmentally regulated homeobox genes Hox2.5, Hoxb3 and Hoxa6, all of which may be involved in neural development, and the gene encoding neuroserpin, a factor that promotes neuronal plasticity.

Of the four genes upregulated by CR in the neocortex which were assigned to the stress response class, two encode I-κ B α chain and I-traf, which act to attenuate stress responses by inhibiting the NF-kB signaling pathway involved in the response to oxidative stress and inflammation in the nervous system (Kaltschmidt *et al*., 1999). CR also upregulated genes which modulate the inflammatory response such as *Ia2* and *Ia5* (encoding interferon α isoforms 2 and 5) and Relb, a gene encoding a transcription factor which may suppress inflammation through NF-κB-mediated signaling (Ryseck *et al*., 1996).

CR was associated with a reduction of 1.7-fold or greater in the expression of 84 genes in the neocortex. The largest functional class (11%) consisted of genes involved in protein synthesis. Inflammatory related transcripts downregulated by CR include the proinflammatory cytokine Mcp5. A state of lowered endogenous oxidative stress in CR mice is also suggested by reduced expression of the chaperone-encoding genes Hsp27 and Cypa, and also the genes encoding the p65 and p100 subunits of NF-kB. Taken as a whole, these results suggest that metabolic alterations induced by CR may profoundly affect brain aging.

Table 3 Global view of transcriptional changes induced by aging and CR in the mouse brain

Aging[a]	CR
↑ Inflammatory response Induction of: Complement cascade MHC molecules Microgial activation factors Inflammatory peptides	↑ Growth and trophic factors Induction of: Developmentally regulated genes Neurotrophic support
↑ Stress response Induction of: Heat shock factors Oxidative-stress inducible genes Lysosomal proteases	↑ DNA synthesis Nucleotide precursors DNA replication factors
↓ Protein turnover Suppression of: Ubiquitin-proteasome pathway	↓ Protein synthesis tRNA synthesis Elongation factors
↓ Growth and trophic factors Developmentally regulated genes Neural plasticity	↓ Stress response Suppression of: Oxidative-stress response NF-κ-B signaling Heat shock factors

[a]Aging changes apply to both the neocortex and cerebellum. The study of the effect of CR was limited to the neocortex (Lee *et al.*, 2000).

The gene expression profile of aging and CR in rhesus monkey vastus lateralis muscle

We recently reported the effects of aging and adult-onset CR on the gene expression profile of 7070 genes in the vastus lateralis muscle from rhesus monkeys (Kayo *et al.*, 2001). The average lifespan of rhesus monkeys in our colony is ~27 years and the maximum lifespan is ~40 years. Gene expression analysis of old rhesus monkeys (mean age 26 years) was compared with young animals (mean age 8 years). Aging resulted in a selective upregulation of transcripts involved in inflammation (a class not observed to be upregulated in the mouse gastrocnemius muscle) and oxidative stress, and a downregulation of genes involved in mitochondrial electron transport and oxidative phosphorylation. Middle-aged monkeys (mean age 20 years) subjected to CR since early adulthood (mean age 11 years) were studied to determine the gene expression profile induced by CR. CR resulted in an upregulation of cytoskeletal protein encoding genes and also a decrease in the expression of genes involved in mitochondrial bioenergetics. Surprisingly, we did not observe any evidence for an inhibitory effect of adult-onset CR on age-related changes in gene expression. These results indicate that the induction of an oxidative-stress-induced transcriptional response may be a common feature of aging in skeletal muscle of rodents and primates, but the extent to which CR modifies these responses may be species-specific. However, critical differences in the experimental design of our mouse and monkey studies may explain the lack of efficacy of CR in retarding age-associated transcriptional changes in monkeys. Most notable are the early life onset of CR in the mouse study and the study of truly old CR mice (versus middle age CR monkeys) in the mouse study.

Implications for aging research

These data underscore the utility of high-density oligonucleotide microarray gene expression analysis in the study of complex biological phenomena such as the aging process. Further, gene chips are useful tools for evaluating the impact of nutritional interventions by providing a global, unbiased view of gene expression alterations. The promise of this approach derives from the ability to provide (eventually) a genome-wide screen within a single experimental setup. Proteomics is expected to offer similar opportunities.

However, certain limitations are associated with data generated by microarrays. First, the observed collection of gene expression alterations in aging whole tissues such as muscle and brain is complex, reflecting the presence of diverse cell types. Second, the ability of oligonucleotide microarrays accurately to measure mRNA levels for thousands of genes has predated the development of statistical tools to minimize the occurrence of false positives and false negatives. Additionally, changes in mRNA levels may not always result in a parallel alteration in protein levels.

None the less, the complete or partial prevention of the majority of the observed aging alterations by CR in mouse muscle and the targeted prevention of stress response and inflammatory transcripts in the brain provide biological validation for this approach. Further, these data suggest that gene expression patterns can be used to assess the biological age of a tissue. Extension of our study to other organs should result in the identification of hundreds of tissue-specific biomarkers of aging, facilitating the elucidation of aging mechanisms and the development of interventions.

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