Age-Associated Changes in Gene Expression Patterns in the Liver

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Aging is one of the least clearly understood biological processes. Alteration of oxidation/reduction (redox) enzymes has been demonstrated with aging; however, a systematic analysis of expression patterns has not been performed. The liver plays a key role in homeostasis and detoxification; therefore alteration of hepatic gene expression with aging may affect outcome after surgery. The purpose of our study was to assess changes in gene expression patterns in aged livers from both rats and humans using gene array analysis. Total RNA was extracted from young (2-month-old) and aged (2-year-old) rat livers, as well as young (1-year-old) and aged (78-year-old) human livers. Gene expression patterns were compared using Affymetrix GeneChip arrays. The expression pattern of selected genes was confirmed by reverse transcription–polymerase chain reaction. A threefold or greater change in gene expression was noted in 582 genes in the aged rat livers and 192 genes in the aged human livers. Comparison of the genes that were increased with aging demonstrated some similar patterns of expression in the rat and human livers, particularly in members of the antioxidant family and the cytochrome P-450 genes. Our findings demonstrate changes in the expression pattern of genes in the liver with aging. Concomitant increases in the expression of important antioxidant and detoxifying genes were noted in the livers of both rats and humans. This induction pattern suggests a complex link between changing hepatic detoxification/redox capability and senescence. (J GASTROINTEST SURG 2002;6:445–454.) © 2002 The Society for Surgery of the Alimentary Tract, Inc.

KEY WORDS: Aging, gene expression, liver, gene array

Medical advances in the past century have enabled persons in our society to live longer and remain healthy for a longer period of time. It is estimated that the number of elderly will continue to increase and by the year 2050, nearly 25% of Americans will be at least 65 years of age.[1] As the number of persons reaching advanced age increases, there will be an associated need for increased surgical care. From 1980 to 1996, the percentage of operations that were performed in patients over 65 years of age increased from 19% to 36%.[2] Furthermore, it is estimated that at least 50% of patients in most general surgery practices are over 65 years of age.[3] With aging comes a decline in physiologic function in most organ systems.[4] In normal, nonstressful conditions, this decline usually has minimal consequences; however, with stress (e.g., surgery or trauma), the ability of the aged patient to respond to these increased demands is seriously compromised.[5] Therefore it is imperative that all physicians recognize and understand the changes that occur in various organ systems with aging, if we are to achieve improvements in the care of elderly patients.

Aging, a universal phenomenon of all organisms, is one of the least clearly understood biological processes. There are currently a number of theories proposed to explain the changes associated with aging.[6] Oxidative damage to mitochondrial DNA is thought to play a role in aging and in various degenerative processes.[7] This theory, often called the free-radical theory of aging, provides a conceptual approach to the study of aging.[8–10] Free radicals are reactive oxy-

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gen species (ROS) that are normally generated at low levels in all living aerobic cells by the respiratory chain of the mitochondria. The accumulation of ROS is thought to damage cellular machinery and contribute to the changes seen with aging.[8] To oppose this internal threat, cells have a number of antioxidant defense mechanisms including antioxidant enzymes such as superoxide dismutase (SOD), which catalyzes the dismutative reaction between two superoxide molecules and hydrogen to form H_2O and O_2.[11] Other antioxidants include the glutathione S-transferase (GST) enzymes, which eliminate ROS through conjugation reactions and NADH–cytochrome b_5 reductase, which reduces the membrane antioxidant coenzyme Q.[12] These enzymes provide a scavenging system to eliminate potentially harmful oxygen metabolism byproducts, but it is estimated that the average human cell still encounters approximately 10,000 free-radical exposures a day.[13]

The liver is a critical organ in protection from oxidative damage.[14] In addition, the liver plays a major role in the breakdown of potentially toxic lipophilic toxins through the action of oxidation and reduction enzymes such as the cytochrome P-450 enzymes.[15] This family of enzymes adds hydroxyl groups to a number of toxins, which facilitates their excretion in the urine.[16] Age-associated changes in liver physiology and function have been described.[17] With aging, there is a general decrease in organ size and hepatocyte number. Liver enzymes are routinely unaltered with senescence; however, during periods of stress, the liver in the aged patient may not be able to meet the demands of hypermetabolic states. Elucidating the changes in the expression of genes in the liver with aging may lead to a better understanding and treatment of hepatic dysfunction after surgery or major stress. Therefore the purpose of our study was to assess age-associated changes in gene expression patterns in rat livers and compare them with changes in human livers. We used gene array technology for these studies. Genomic expression profiling with high-density oligonucleotide arrays represents a powerful tool to expand current molecular studies and provide a global assessment of changes in gene expression patterns associated with aging.

MATERIAL AND METHODS

Total RNA was isolated using Ultraspec II (Biotex, Houston, TX). GeneChip oligonucleotide glass-slide, high-density arrays and hybridization protocols were obtained from Affymetrix (Santa Clara, CA) (rat array: RG_U34A = 8,797 gene probes; human array: HG_U95A = 12,626 gene probes). In addition, Affymetrix GeneChipSuite 3.3 expression analysis software and Affymetrix EASI databases were used. Decision Site Gene Array Explorer software was also purchased. Polymerase chain reaction (PCR) reagents were purchased from GIBCO-BRL (Grand Island, NY), and PCR primers were synthesized by Oligos Etc. (Wilsonville, Ore.). For the GST activity assay, the substrate, 1-chloro-2,4-dinitrobenzene, and glutathione were purchased from Sigma Chemical (St. Louis, MO). All other reagents were of molecular biology grade and were purchased from either Sigma or Amresco (Salon, OH).

Animals and Tissue Preparation

Young (2-month-old) and aged (24-month-old) male Fischer 344 rats were obtained from the National Institute on Aging (Bethesda, MD) stock colony maintained under barrier-reared conditions. Aged animals were defined as those that had achieved the age at which one-half of the population ordinarily dies (median survival time). In humans that age is approximately the eighth decade of life; in Fischer 344 rats the age is 23 to 26 months.[18] After receipt, rats were housed at a constant temperature of (22° C) with 12-hour light-dark cycles. Rats were fed standard rat chow (Ralston Purina, St. Louis, MO) ad libitum.

At the end of a 7-day acclimatization period, the rats were anesthetized with 5% halothane and then killed by decapitation. The entire liver was removed, immediately frozen in liquid N_2, and maintained at −70° C until RNA extraction. Two human liver samples from a 1-year-old male (young) and a 78-year-old male (aged), both of whom sustained intracranial injuries, were purchased from Tissue Transformation Techniques (Edison, NJ). Tissue samples were stored at −70° C until extracted for RNA. Tissue acquisition and subsequent use were approved by the Institutional Review Board at The University of Texas Medical Branch.

RNA Isolation and Affymetrix GeneChip Labeling Analysis

For both rat and human samples, total RNA was extracted by the method of Chomczynski and Sacchi[19] using Ultraspec RNA in accordance with the manufacturer's instructions.

Using total RNA (25 to 30 μg), first-strand cDNA was synthesized with a T7 - $(dT)_{24}$ oligomer (5′ GGCCAGTGGAATTGTAATA CGACTCACTA-TAGGGAGGCGG-dT_{24} 3′) and SuperScript II reverse transcription (RT; Life Technologies, Rock-

ville, MD). Second-strand synthesis converts the cDNA into the DNA template needed for in vitro transcription. During in vitro transcription, target RNAs (cRNA) were biotin labeled with bacteriophage T7 RNA polymerase-directing synthesis. The biotin-labeled cRNAs then were fragmented to a mean size of 150 bases. The samples were tested with hybridization to test chips containing several housekeeping genes (e.g., actin, GAPDH).

Hybridizations were performed at 45° C for 16 hours in 0.1 mol/L MES (pH 6.6), 1 mol/L NaCl, 0.02 mol/L EDTA, and 0.01% Tween 20. Four prokaryotic genes (*bio* B, *bio* C, *vio* D from *E. coli* biotin synthesis pathway, and *cre*, the recombinase gene from P1 bacteriophage), were added to the hybridization cocktail as internal controls. These control RNAs were used to normalize expression levels between experiments and, because they are added at varying copy number (*Bio* B, 1.5 pmol/L; *Bio* C, 5 pmol/L; *Bio* D, 25 pmol/L and CRE, 100 pmol/L), they were used in estimating relative abundance of RNA transcripts in the sample. The arrays (rat: RG_U34A, 8,799 genes; human HG_U95A, 12,626 genes) were washed with nonstringent (1 mol/L NaCl, 25° C) and stringent (1 mol/L NaCl, 50° C) solutions. Staining was completed with phycoerythrin streptavidin (10 mg/ml final). The GeneChips were then scanned with a Hewlett-Packard scanner and analyzed. Further data analysis was performed using the Affymetrix EASI database (to assign gene descriptions to query probe sets) and the Spotfire Decision Site software system (Somerville, Mass.).

Reverse Transcription–Polymerase Chain Reaction

For RT-PCR, the sequences of the specific primers used for the reactions were as follows: rat NADH–cytochrome b5 reductase, (sense) 5′ ATCCCAAGT TTCCAGCTGGAG-3′ and (antisense) 5′-CCAGTACAGCTGGATGACCAG-3′; rat Cu/Zn SOD, (sense) 5′-GGCGGCTTCTCTCGT CTCCTTG-3′ and (antisense) 5′CAGCAGATGATGAGTCTGAGACTCAG-3′; human cytochrome P-450 1A1 (TCCD inducible), (sense) 5′CAGTAATGGTCAGAGCATGTCC-3′ and (antisense) 5′-GGTTCACACCAAATACATGAGGCT-3′; human cytochrome P-4502C18 (CYP2C18), (sense) 5′-GGTAACAAAGACTTGGAATCC-3′ and (antisense) 5′-GCAGGAGTCCATATCTCAG-3′; and glyceraldehyde-3-phosphatase dehydrogenase (GAPDH), (sense) 5′-TCCACCACCTGTTGCTGTA-3′ and (antisense) 5′-ACCACAGTCCATGCCATGCCATCAC-3′.

The RT reaction was performed according to the manufacturer's instructions (GIBCO-BRL) and as previously described.[20] Briefly, a 50 μl reaction consisting of 5 μg total RNA, 1 μl oligo (dT) and RNase free water was incubated for 10 minutes at 70° C. The mixture was then mixed with 5× buffer, 0.1 mol/L DTT, and 10 mmol/L dNTP mix and incubated for 2 minutes at 42° C. This was followed by the addition of SuperScript II (200 U/μl) and further incubated for 50 minutes at 42° C. The reaction was halted with a temperature increase to 70° C for 15 minutes. A parallel reaction without the addition of RNA was performed to ensure lack of contamination.

After the completion of the RT reactions, 1 μl of cDNA template was used for PCR. Reaction mixtures contained 1 μl of cDNA, 5 μl of 10× PCR buffer, 1.5 μl of 50 mmol/L MgO_2, 1 μl of 10 nmol/L dNTP, and 1 μl of 10 μmol/L sense and antisense primers. Autoclaved-distilled H_2O was added to a total volume of 50 μl. Reactions were mixed and covered with sterile mineral oil, and PCR was initiated with 95° C hot-start addition of Taq DNA polymerase and carried out according to the following profile: an initial denaturation for 1 minute at 95° C followed by 25 cycles at 94° C for 55 seconds, 60° C for 55 seconds, and 72° C for 1 minute and 30 seconds. Additional parallel reactions were performed without the addition of cDNA template to ensure lack of contamination. An aliquot of each PCR reaction was resolved by gel electrophoresis with 1% agarose gel containing ethidium bromide. Bands were visualized by ultraviolet illumination.

Glutathione S–Transferase Activity Assay

Total GST activity of cytosolic fractions of protein was assayed using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate as described originally by Habig et al.[21] Frozen liver samples were homogenized in a lysis buffer (0.25 mol/L sucrose, 1 mmol/L EDTA, pH 7.4). Cytosolic fractions were obtained by centrifugation (14,000 rpm for 30 minutes, followed by 45,000 rpm for 60 minutes). Protein concentration was determined by the method of Bradford.[22] Reaction mixtures, consisting of 1.5 ml potassium buffer (0.13 mol/L, pH 6.5), 20 μl CDNB (76 mg/10 ml ETOH), and 10 μl protein sample (1:10 dilution), were analyzed by spectrophotometry at a wavelength of 340 nm after the addition of GSH (76 mg/10 ml H_2O), which initiates enzyme activity.[23] Activity measurements were standardized to protein content and expressed as μmol/min/mg protein.

Statistical Methods

Rat GST activity per minute was analyzed using analysis of variance for a two-factor experiment with

repeated measures on time. The two factors were age group (2 and 24 months) and time (5 points from 1 to 5 minutes). Effects and interaction were assessed at the 0.05 level of significance. Fisher's least significant difference procedure was used for multiple comparisons with Bonferroni adjustment for the number of comparisons.

RESULTS

Age-Associated Changes in the Expression Pattern of Genes in Rat and Human Livers

To determine the effects of aging on the expression pattern of genes in the liver, we extracted RNA from young and aged rat and human livers; gene expression profiles were determined using rat and human microarrays from Affymetrix. Expression changes were reflected in the differences in signal intensity from array hydrizations. Analysis of the microarray differences in the hybridization signals was accomplished with the use of Affymetrix Expression Suite 3.3 software; a threefold or greater change in signal intensity was considered a significant change in expression (Fig. 1). Analysis of expression patterns in aged rat livers demonstrated a threefold or greater change in the expression of 582 genes compared with the livers of the young animals (552 genes were increased and 30 genes were decreased with aging) (see Fig. 1, *A*). Genes with changes of threefold or greater comprised approximately 6% of the probes on the rat RG_U34A microarray, with changes ranging from a 105-fold increase to a 261-fold decrease in the expression of genes in the aged livers.

Analysis of the human (young and old) liver samples demonstrated threefold or greater changes in the expression of 192 genes (approximately 1% of the probes on the human U95A microarray) (see Fig. 1, *B*). In contrast to the rat liver in which the majority of gene changes were noted to be an actual increase, only 43 genes in the aged human liver demonstrated a threefold or greater increase of expression, whereas 149 genes were decreased by threefold or greater. Taken together, our analysis of rat and human livers has identified a subset of genes that appear to be altered with aging. A number of genes were increased threefold or greater, particularly in the rat liver, further emphasizing the fact that gene expression does not remain static or uniformly decline with senescence.

Analysis of the genes with increased expression in the aged rat and human livers demonstrated that a number of these genes are members of the redox and detoxification gene families. These included members of the GST, cytochrome P-450, UDP-glucuronosyltransferase, and SOD families, with increases of expression ranging from three- to 61-fold in the aged rat liver (Table 1). Similarly, increases in members of the GST, cytochrome P-450, and UDP-glucuronosyltransferase gene families were detected in the aged human liver (Table 2).

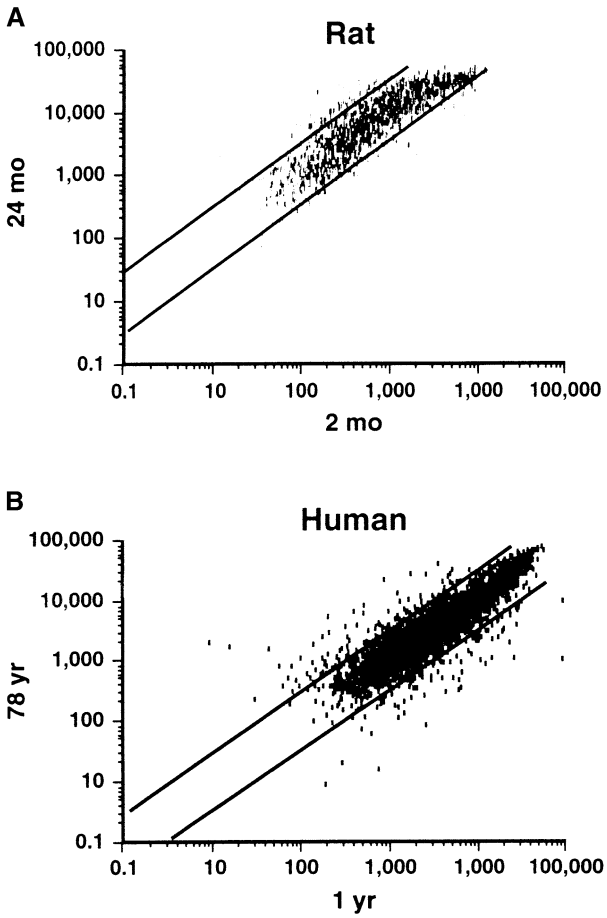

Fig. 1. Gene array hydrization signal intensities. Hydrization differences as calculated and normalized by Affymetrix Expression Suite 3.3 software. **A,** Scatter plot of differential signal intensity between young (2-month-old) and aged (24-month-old) rat livers as analyzed by RG_U34A Affymextrix GeneChip array. **B,** Scatter plot of differential signal intensity between young (1-year-old) and aged (78-year-old) human livers as analyzed by HG_U95A Affymextrix GeneChip array. Normalization for both rat and human experiments included elimination of genes with insufficient hydrization for analysis as determined by Expression Suite 3.3 software.

Confirmation of Gene Expression Changes by RT-PCR

To confirm our findings obtained by microarray analysis, the expression of selected genes was analyzed by a semiquantitative RT-PCR procedure using the same RNA samples that were used in the microarray analysis (Fig. 2). Two genes in the rat that

Table 1. Subsets of genes that are increased in the aged rat liver

Rat genes	Gene bank	Fold increase
UDP-glucoronosyltransferase		
Exon 1	D38065	29
3-Methylchoanthrene-inducible	S56937	20
UGT1	D83796	3
Glutathione S–transferase		
Subunit 13 (GSTK-1)	S83436	61
Yc2 subunit (GSTA5)	S82820	51
Subunit 8	X62660	27
P subunit	X02904	4
Cytochrome P-450 enzymes		
P-450 polypeptide	M14775	13
P-450 2J3 (CYP2J3)	U39943	4
CYP3A1	X62086	4
Antioxidant enzymes		
Cu/Zu SOD	M25157	56
Other		
NADH–cytochrome b5 reductase	D00636	48
Glutathione synthetase	L38615	5
Glutathione reductase	U73174	5

were found to be increased in the aged liver (NADH–cytochrome b5 reductase and Cu/Zn SOD) were analyzed using rat-specific primers that yield PCR products of 660 and 540 bases, respectively; primers for rat GAPDH were added to the same reaction vessels as a constitutively expressed control. In addition, a control reaction using all of the PCR components, with the exception of reverse transcription cDNA, was performed to ensure that no contamination existed. Similar to the microarray analysis, the expression of both genes was increased in aged (24-month-old) livers compared with young (2-month-old) livers; GAPDH was expressed at similar levels in both samples (see Fig. 2, *A*). The expression of two genes noted to be increased in the aged human liver was further assessed. Analysis by RT-PCR demonstrated that both cytochrome P-450 1A1 (2,3,7,8 tetracholordibenzo-p-dioxin [TCDD]-inducible) and cytochrome P-450 2C18 (CYP2C18; clone 6b) were increased in the aged livers compared with the 1-year-old livers (see Fig. 2, *B*). GAPDH was expressed at similar levels in the samples.

To further confirm changes noted by our initial microarray analysis, we analyzed three additional young (2-month-old) and aged (24-month-old) rat livers for expression of NADH–cytochrome b5 reductase and Cu/Zn SOD (Fig. 3). Although the expression varied in the in vivo samples, increased expression (~10-fold) of NADH–cytochrome b5 reductase was noted in the three aged livers compared with the livers from the young rats (see Fig. 3, *A*). An analysis of the expanded group of rat liver samples demonstrated that the results for Cu/Zn SOD are less consistent with no significant change in expression noted in the aged and young livers (see Fig. 3, *B*). These results demonstrate the variability in the expression patterns in vivo and further emphasize the necessity of confirming the data obtained by microarray analyses using more conventional techniques (e.g., RT-PCR) and using samples from different animals.

Table 2. Subsets of genes that are increased in the aged human liver

Human genes	Gene bank	Fold increase
UDP-glucuronosyltransferase		
C19 steroid specific	U59209	3
Glutathione S–transferase		
Ha subunit 2 (GST)	M16594	19
Theta 2 (GSTT2)	L38503	8
Cytochrome P-450 enzymes		
P-450 1A1 (CYP1A1-TCDD-inducible)	K03191	9
CYP1A2	M31667	5
P-450 2C18 (CYP2C18)	M61853	3

Assessment of GST Functional Activity in Young and Aged Livers

We next determined whether the increased expression of members of the GST gene family was associated with increased functional activity. GST activity was assessed in the young and aged rat and human livers by testing the ability of these proteins to conjugate 1-chloro-2,4 dinitrobenzene (Fig. 4). Although not significant, there is a trend toward decreased GST activity in the aged rat liver compared with the young rat liver. A similar trend in GST enzyme activity is noted in human liver samples from the 78-year-old compared with those of the 1-year-old.

Collectively these findings demonstrate alterations in the expression of a number of the antioxidant and detoxification genes in the liver with aging. At least in the case of GST, increased expression of this gene in the aged liver does not appear to correlate with an increase in enzyme activity.

DISCUSSION

Aging is associated with changes in physiologic function and gene expression, which may greatly af-

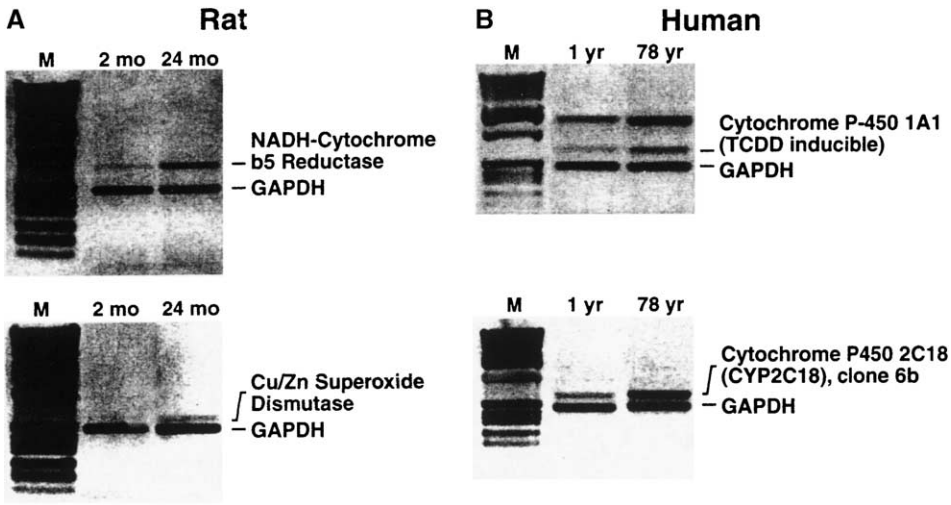

Fig. 2. RT-PCR analysis of rat and human expression patterns. **A,** To confirm rat gene expression increases, RNA extracted from young (2-month-old) and aged (24-month-old) rat livers was analyzed by a semiquantitative RT-PCR with primers for NADH–cytochrome b5 reductase and Cu/Zn SOD. **B,** RNA extracted from young (1-year-old) and aged (78-year-old) human livers was analyzed by a semiquantitative RT-PCR with primers for cytochrome P-450 (1A1) and cytochrome P-450 (2C18). The integrity of the RT reaction was confirmed by amplification using primers to the constitutively expressed GAPDH gene. Control experiments consisted of parallel reactions with all of the RT-PCR components except for the reverse transcription cDNA. M = molecular weight marker.

fect the functional responses of elderly patients after trauma or major operative procedures.[24] In our present study, we have used gene array techniques to analyze changes in the expression patterns of genes in the liver associated with aging. Increased expression of redox and detoxification enzymes, including the GST, UDP-glucuronosyltransferase, and cytochrome P-450 enzyme families, were noted in both aged rat and aged human liver samples. Other genes displaying increased expression in the rat liver included the antioxidant enzymes Cu/Zn SOD and NADH–cytochrome b5 reductase. These increased expression patterns were further corroborated by RT-PCR analysis of selected genes. Our findings demonstrate changes in the expression patterns of similar gene families in aged rat and aged human livers and suggest that gene array techniques will be useful to better delineate the changes in gene patterns associated with senescence.

The development of new technologies arising from the human genome project, such as DNA microarray analysis, will have an enormous impact on the future studies of normal cellular processes as well as disease states. The array-based methods, which involve the mobilization of thousands of cDNAs on a solid matrix, have the advantage of a high-output, direct, and rapid readout of hybridization results and immediate information on the expression patterns of numerous genes. Recent studies have used microarray techniques to analyze changes in gene expression patterns with aging. Lee et al.[25] analyzed skeletal muscle from mice of different ages and noted that 113 of 6347 genes displayed a greater than twofold change in expression levels as a function of age. Stress-related genes were noted to be increased, whereas genes involved in energy metabolism, biosynthetic pathways, and protein turnover were decreased with aging. In another study by Ly et al.,[26] gene expression patterns were measured in actively dividing fibroblasts isolated from young, middle-aged, and elderly humans and humans with progeria, a rare genetic disorder characterized by accelerated aging. Except for some common changes in expression patterns of the stress-responsive genes, there was little overlap between the alterations noted in these two studies,[25,26] suggesting that specific tissues may respond to aging differently. Taken together, our present study, as well as those of others,[26–30] confirm the fact that aging is associated with changes (either increases or decreases) in gene expression patterns. These changes are highly dependent on tissue type and species.

The accumulation over time of ROS, which are byproducts of metabolism and produced in all aerobic organisms, is thought to play a role in the pathogenesis of certain diseases and contribute to the changes noted with aging.[7,8,31,32] Antioxidants are "free-radical scavengers" that detoxify these potentially toxic byproducts;[6] alterations in the activity of

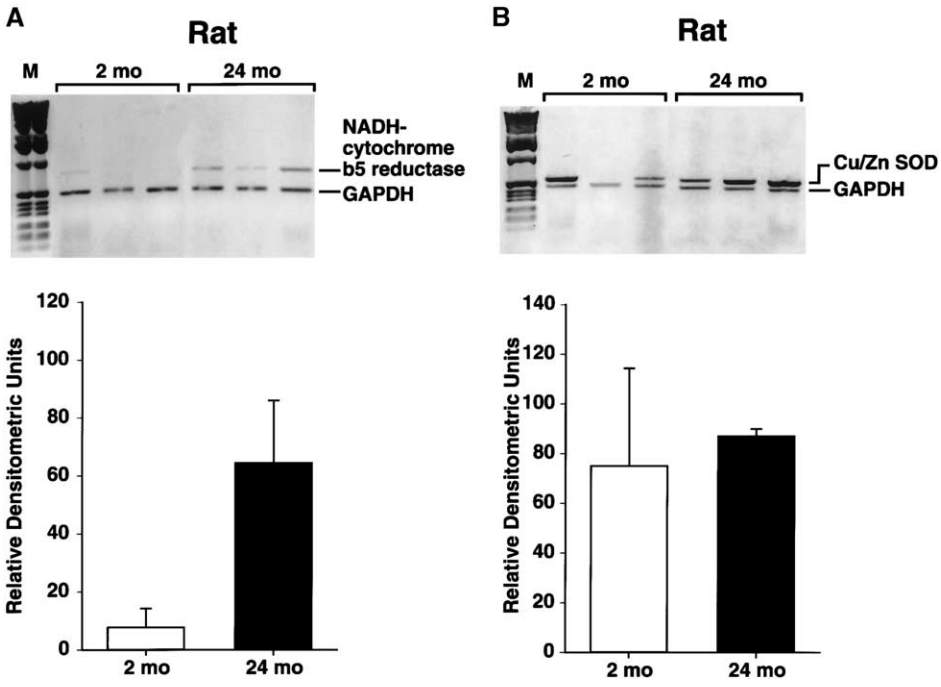

Fig. 3. RT-PCR analysis of additional in vivo young and aged rat livers. **A,** RNA was extracted from three additional young (2-month-old) and aged (24-month-old) rat livers. Samples were analyzed by RT-PCR with primers for NADH–cytochrome b5 reductase (**A**) and Cu/Zn SOD (**B**). Primers for the constitutively expressed GAPDH gene were run in the same reactions. RT-PCR results were quantitated using scanning densitometry and expressed as relative densitometric units after normalizing for GAPDH (mean ± SEM).

various antioxidants may allow for ROS accumulation. In our present study we have focused on changes that occur in the redox gene families in the liver. We found increased expression of several antioxidant enzymes including the members of the GST family, Cu/Zn SOD, and the membrane antioxidant NADH–cytochrome b5 reductase. To correlate expression patterns in the rat with changes in the human, we have assessed liver samples from an infant and an elderly patient and found, similar to that which occurs in the rat, an increase in the expression of members of the GST family. Although a powerful method to rapidly and simultaneously assess the expression changes of a multitude of genes, this technique represents a "snapshot" depicting changes in gene expression in a single tissue sample at a single time point. A number of factors may influence the expression patterns noted in an array analysis. Environmental factors including differences in diet, stress, and manner of tissue collection may alter the expression patterns detected by a single array. Therefore it is imperative that the changes noted by gene arrays be confirmed by more conventional techniques and, if possible, in tissues examined from multiple subjects. We have used RT-PCR to confirm changes noted in selected genes in both rat and human livers. Moreover, we have analyzed additional rat livers by RT-PCR to confirm changes in different animals of the same age. Of note, increased expression of NADH–cytochrome b5 reductase was consistently noted in aged livers compared to the young samples. In contrast, although the expression of Cu/Zn SOD was increased in the aged rat liver by gene array analysis, there was no difference in the expression pattern when other liver tissues were analyzed by RT-PCR. These studies further emphasize the importance of confirming the changes noted on gene arrays by other more conventional methods and also the need to analyze different tissue samples given the inherent variability found in individual in vivo specimens.

To further analyze, on a functional level, age-associated changes in GST, we measured GST enzyme activity in rat and human livers. Despite an apparent increase in gene expression in the aged liver, GST enzyme activity was not significantly altered in rat and human livers, although there was a trend toward decreasing GST activity in the aged livers compared with the young samples. Other studies have demonstrated a significant decrease in antioxidant enzyme activity with aging.[29,33,34] For example, both Rao et al.[29] and Semsei et al.[33] have shown a decrease in the antioxidant enzymes Cu/Zn SOD and catalase in rat livers with aging. However, Carrillo et al.[34]

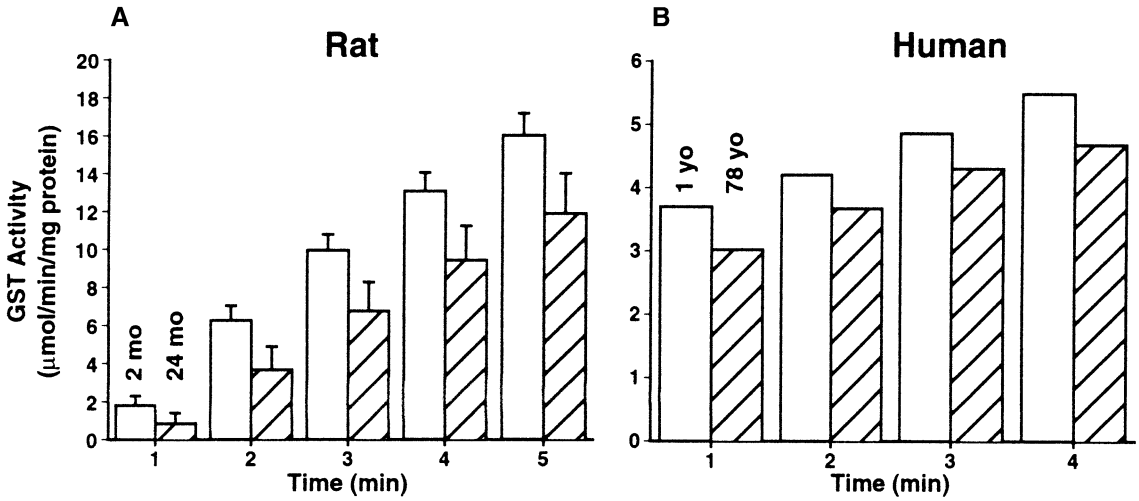

Fig. 4. Glutathione S–transferase activity. **A,** Cytosolic protein fractions from four young (2-month-old) (*open bars*) and four aged (24-month-old) (*single-hatched bars*) rat livers were analyzed by spectrometry for the ability of glutathione S–transferase *(GST)* to conjugate 1-chloro-2, 4-dinitrobenzene (CDNB) activity. Results are expressed in μmol/min/mg of protein sample (mean ± SEM). **B,** Cytosolic protein fractions from young (1-year-old) (*open bars*) and aged (78-year-old) (*single-hatched bars*) human samples analyzed by spectrophotometry for GST activity (μmol/min/mg protein sample).

found that, despite a decrease in catalase activity in aged male rats, catalase activity in aged female rats was increased compared with that in young rats. Similar differences in catalase activity have been described by Rikans et al.[35]

In humans the activities of the antioxidant enzymes Cu/Zn SOD, glutathione peroxidase, glutathione reductase, and GST were measured in individuals from 1 month to 63 years of age. An inverse correlation between age and the enzyme activities of Cu/Zn SOD, glutathione reductase, and GST was noted; however, in contrast, glutathione peroxidase activity increased with aging.[36] These findings suggest that there is a variable change in the antioxidant family members with aging. That is, a general decline in the activities of the antioxidants is not a universal phenomenon of aging and appears to be dependent on the particular enzyme analyzed. Gene array studies will further assist in characterizing the changes in a number of different gene families, which will then need to be assessed at the functional level. In our current study we have looked at two extremes of aging. It will be important to analyze intermediate-age groups in the future to determine whether a gradient of expression changes occur with aging or, conversely, if a certain age must be achieved for these alterations in expression to occur. In addition, an important question to address in the future is the effect of stress in the elderly on the activity of these enzymes.

Members of the cytochrome P-450 family of genes were noted to be increased in the aged rat and human livers. These enzymes serve to detoxify lipophilic compounds into water-soluble metabolites by oxygenation reactions. In addition, other functions of members of this family include hydroxylation of steroids and biotransformation of drugs.[37] Although our findings suggest an increase in the expression levels of these genes, we have not assessed the functional activity of these enzymes with aging to determine whether the enzyme activity correlates with actual gene expression levels. This is another important aspect of gene array studies that must be considered. That is, gene expression changes may not correlate with protein expression or functional activity in the cell. Therefore the significance of expression changes noted by gene array analysis should be interpreted with caution with regard to their actual consequences in the cell.

CONCLUSION

Our results, obtained by gene array analysis, provide insights into the changes in gene expression patterns associated with the aging process. Further, our results demonstrate similar increases in the expression of antioxidant genes, such as GST and NADH–cytochrome b5 reductase, in the livers of aged rats and humans. The expression of members of the cytochrome P-450 family of genes was also increased both in rats and humans, suggesting common regulatory pathways for age-associated changes in the

mammalian liver. Delineating the gene expression profiles that are altered with aging will provide a better understanding of the functional changes that occur in various organs with senescence. This improved understanding may provide novel approaches to the treatment of aged patients.

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Discussion

Dr. S. Strasberg (St. Louis, MO): I am really not qualified to ask a question about this study except in terms of experimental design. I find the age selection that you used to be somewhat unusual and, as a result, I find it difficult to see how you arrived at your conclusions. You have chosen to study two persons: one, a very young human who is in development, and the other, a very old human. If you wanted to make the point that genes get turned on with aging, it would seem to me that you need persons of other ages. I think a young adult (i.e., a fully mature human) should be used for comparison to very old patients rather than an infant if one is to conclude that genes are turned off with aging. This study could likewise be interpreted to show that certain genes are turned off during development.

Dr. R. Thomas: That is a very good point. We would like to expand our studies and perform more gene array experiments using rat and human samples of different ages. We chose to use our array data as a "snapshot" of expression changes; therefore our samples were of young and aged subjects. I think the point is valid that this is not a comprehensive look at the total expression changes throughout development of either rats or human.

Dr. M. Sarr (Rochester, MN): First of all, congratulations on using RT-PCR to confirm the chip analysis; however, your N value here is only 1 for the humans, and I am sure you have been expecting this question. You are making fairly broad generalizations based on one liver in a child and one liver in an adult. That is potentially dangerous.

Dr. Thomas: I agree. Again, we understand that this is a limited number of samples. We plan to extend these findings. This experiment was a starting point.

Dr. J. Cullen (Iowa City, IA): A number of the genes that you are looking at or the antioxidants that you are looking at are actually induced or increased because of an increase in ROS. In aging there is an increase in ROS because there is actually a decrease in the major antioxidant enzymes, manganese SOD, copper zinc SOD, catalase and glutathione peroxidase. Have you looked at the changes either in the immunoreactive protein or the activity of those major antioxidant enzymes?

Dr. Thomas: We did not look specifically at the enzyme function of manganese SOD, Cu/ZN SOD, catalase, or glutathione peroxidase. Our gene array demonstrated increases in Cu/ZN SOD; however, subsequent RT-PCR studies identified variable results.

Furthermore, your point is well taken. Although we confirmed our expression changes by RT-PCR, work is still needed to complete these investigations at the protein and enzyme function level.

Dr. S. Ashley (Boston, MA): This is a nice study. I might have been more interested in what got turned off with age and what protective mechanisms were lost with UCVC. Was there anything that was consistent?

Dr. Thomas: We have not completely investigated the genes that demonstrated a decrease with aging. The decision to investigate genes that increase with aging was made because a general conception of the aging process is that there is a general decline in gene expression and physiologic function.

Invited Discussion—Expert Commentator

Henry A. Pitt (Milwaukee, WI): The paper by Dr. Thomas et al. from Galveston explored the effects of aging on gene expression patterns in the liver. As usual, Dr. Evers and his colleagues have brought the latest research techniques to a surgical meeting. This gene chip technology is a very powerful tool for identifying differences between groups or, in this case, over time, a long time, with aging.

One of the disadvantages, however, is that each study provides more data than can be easily assimilated. As a result, years of follow-up work are required after a gene chip analysis. Another concern about a study like this one we hear today is that large amounts of data are gathered from very few tissue specimens. Therefore, the question arises as to the generalizability of the information.

Another issue is whether data from the liver apply to other organs. In addition, although data are available from this study in over 9,000 rat and 13,000 human genes, this sample is only a small percentage of all the rat and human genome. Therefore, the choice of genes on a chip does limit the conclusions that can be drawn from an individual analysis, even with years of follow-up.