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Effects of Aging and Advanced Glycation on Gene Expression in Cerebrum and Spleen of Mice[1]

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Objective To analyze the effects of aging or advanced glycation on gene expression in the cerebrum and spleen of female C57BL/6J mice. **Methods** The gene expression profile was determined by using cDNA expression arrays containing 588 cDNA. **Results** Aging and advanced glycation resulted in differential gene expression patterns of cerebrum and spleen compared with young mice. Among the 80 genes detected in cerebrum, 43 exhibited a change in mRNA ratios with aging or treatment. Thirty-four changes (79%) were common in aged and D-galactose treated mice, whereas the cerebrum from aged and AGE-lysine treated mice showed common changes in expression of 38 genes (88%). Of the 86 genes detected in spleen, 29 (34%) displayed an age-related decrease in expression, whereas 3 (3%) displayed an increase in expression levels with aging. Eighteen genes from the detectable genes exhibited expression changes in both cerebrum and spleen of mice. **Conclusions** The gene expression profiles of D-galactose and AGE-lysine treated mice resemble those of aged mice. Use of cDNA hybridization arrays may provide a promising tool to explore the mechanism of aging at a molecular level.

Key words: Aging; Galactose; Advanced glycation; Gene expression; DNA microarrays; Cerebrum; Spleen

INTRODUCTION

It is known that reducing sugars can react with proteins and nucleic acids without enzymes aiding to form a series of stable covalent adducts named advanced glycation endproducts (AGE). The nonenzymatic glycation reactions with proteins begin when amino groups react with aldehydes-reducing sugars to form Schiff bases. Unstable Schiff bases can undergo Amadori rearrangements to form more stable but reversible products, and then the Amadori products can undergo over time a series of dehydrations and rearrangements to form irreversible AGE. AGE accumulation contributes to cross-linking of macromolecules such as proteins, to alterations in cellular signaling and to changes in cell-matrix interactions. Nonenzymatic glycation is correlated to the pathogenesis of many age-related pathologies such as arteriosclerosis, Alzheimer's disease, diabetes, nephropathy, and infection[1]. In the 1980s, Cerami *et al.* proposed the hypothesis of nonenzymatic glycation inducing aging.

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They postulated that AGE-mediated cross-linking of long-lived proteins was related to age-associated decline in the functions of cells and tissues with aging, and the elevated level of AGE *in vivo* might accelerate aging process of animals and human beings[2]. Various theories concerning the mechanisms of senescence were developed, and in general the causes of aging could be associated with genetic or environmental aspects. Senescence might be involved in activation or repression of a series of genes. Interactions among the products of these genes might result in aging. In this study, to provide some analysis of the molecular events associated with AGE treatment in mammals, we employed cDNA hybridization arrays to determine the gene expression profile of the aging or AGE-treated process in the cerebrum and spleen of female C57BL/6J mice. Five hundred and eighty-eight genes were immobilized on the Atlas cDNA Expression Array including cell cycle regulators, growth regulators, intermediate filament markers, apoptosis, oncogenes, tumor suppressors, DNA damage response/repair/recombination, cell fate/development regulators, receptors, cell adhesion/motility, angiogenesis regulators, invasion regulators, cell-cell interactions, growth factors, and cytokines.

MATERIALS AND METHODS

Reagents

D-galactose, L-lysine, and bovine serum albumin (BSA) were purchased from Sigma. AGE-modified bovine serum albumin (AGE-BSA) was prepared as described[3]. Rabbit anti-AGE-RNase antibody was provided by Dr. Bucala R. (Picower Institute). Alkaline phosphate conjugated goat antibody directed against rabbit IgG was purchased from Beijing Zhongshan Biotechnology Company. Atlas™ cDNA Expression Array was from Clontech.

Advanced Glycation Endproducts Preparation

AGE-lysine was prepared by incubating 0.78 mol/L L-lysine with 0.63 mol/L D-galactose in phosphate-buffered saline (PBS) at 37℃. After 56 days, the solution became dark brown. The AGE level was 75.6 U/mL as measured by an AGE-specific ELISA[4].

Animals and Treatment

Female C57BL/6J mice were obtained from Laboratory Animal Center, Chinese Academy of Medical Sciences (CAMS). They were randomly divided into 4 groups, 8 mice per group. Groups Ⅰ to Ⅲ were 5-month-old young mice (20-22 g) and group Ⅳ was 20-month-old aged mice (26-28 g). After a 7-day adaptation period, the animals were given daily subcutaneous injections for 56 days following the treatment scheme as shown below:

Group Ⅰ: 0.4 mL PBS as vehicle control,

Group Ⅱ: D-galactose at 50 mg/kg,

Group Ⅲ: AGE-lysine at 50 mg/kg (tail vein injection),

Group Ⅳ: 0.5 mL PBS as old control.

Mice were sacrificed at the end of treatment. Tissues and organs were immediately collected for experiments or stored at -70℃.

Spleen Cell Suspension Preparation

The spleens were removed aseptically after the animals were sacrificed. Single cell

suspensions were prepared by rubbing the tissue against sterile stainless steel wire mesh (100 μm) in 10 mL PBS. Contaminating erythrocytes were lysed by treating cell pellets with 5 mL Tris-ammonium chloride lysis buffer (0.18 mol/L NH_4Cl in 0.17 mol/L Tris, pH 7.2) for 3 min at room temperature. After washing twice with PBS, the lymphocytes were resuspended at 10^7 cells/mL.

RNA Isolation, Probes Preparation and cDNA Array Hybridization

Total RNA was extracted from lymphocytes or frozen tissues, reversely transcribed and used to synthesize [α-^{32}P]dATP-labelled cDNA, and hybridized to the gene chip as described[5]. After hybridization, the hybridization solutions were removed and the gene chips were installed in a fluidics system for washing. Following washings, radioactivity was recorded, and data collected from scanned images were used for analysis.

RESULTS

Effects of Nonenzymatic Glycation on Gene Expression Profile of Cerebrum

The cDNAs prepared from cerebrum of young mice, D-galactose treated and AGE-lysine treated mice and old mice were hybridized to identical membrane arrays. Of the 588 genes surveyed, 80 were detectable to an expression level superior to background. Compared with the young mice, the cerebrum from D-galactose treated, AGE-lysine treated mice and aged mice showed altered expression of 43 genes, and 34 changes were common in the three groups (Fig.1). Different changes of gene expression between aged and D-galactose treated mice accounted for 11.3% (9/80), including MAPKK1, MCH-6, MCH-4, PIG7, ABLL, Wnt-5a, EMMPRIN, T-PA and collagen type XI pro-alpha-2, the major classes of which were apoptosis and invasion regulators. 6.3% (5/80) of genes displayed different expression changes between aged and AGE-lysine treated mice. They were MCH- 6, MCH-4, PIG7, ABLL and collagen type XI pro-alpha-2, and the largest class consisting of genes was involved in apoptosis. Of the 80 genes detectable, 48% (38/80) and 45% (36/80) decreased, whereas 6% (5/80) and 9% (7/80) increased in expression in the cerebrum of D-galactose and AGE-lysine treated mice compared with young controls, respectively.

Effects of Aging on Gene Expression Profile of Splenic Lymphocytes

The cDNAs prepared from splenic lymphocytes of young and old mice were hybridized to identical membrane arrays. Of the 588 genes surveyed, 86 were detectable to an expression

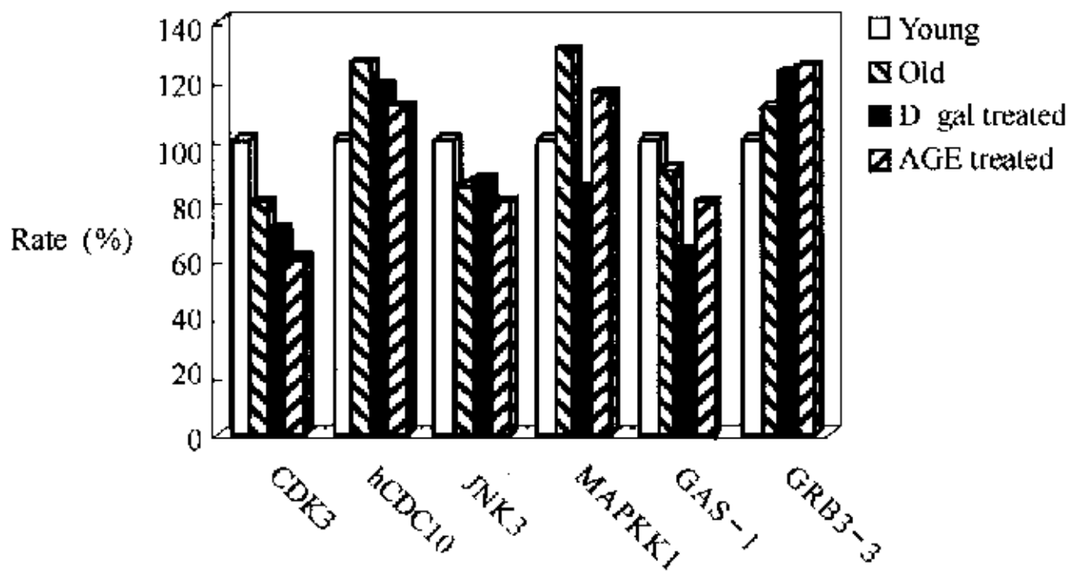

FIG. 1-1. Expression of cell cycle/growth regulators.

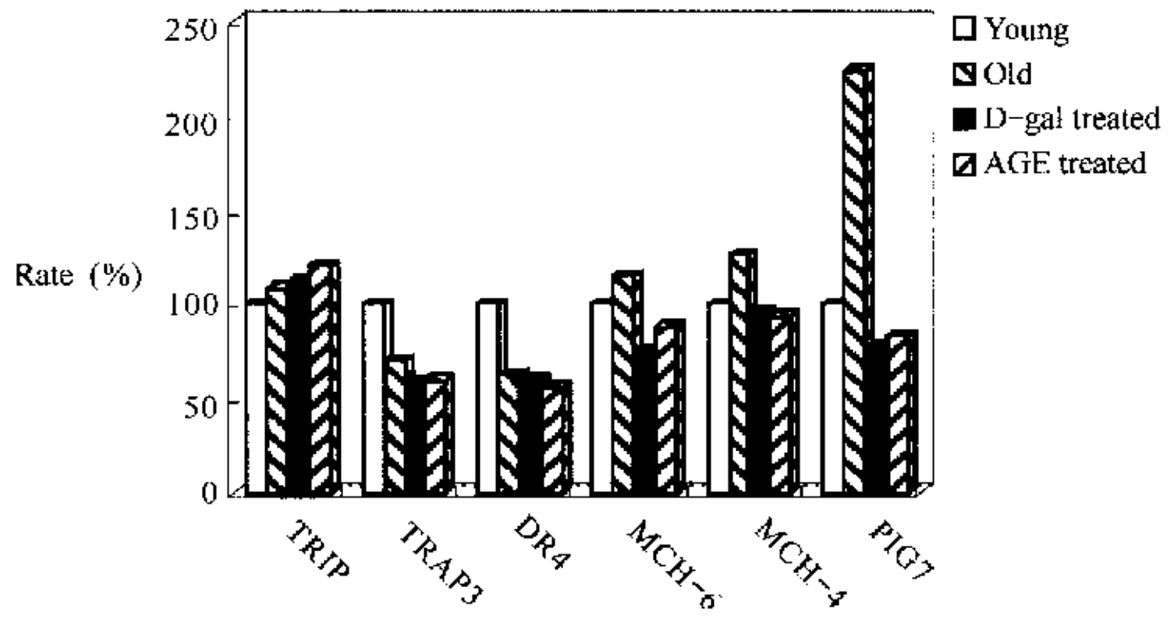

FIG. 1-2. Expression of apoptosis genes.

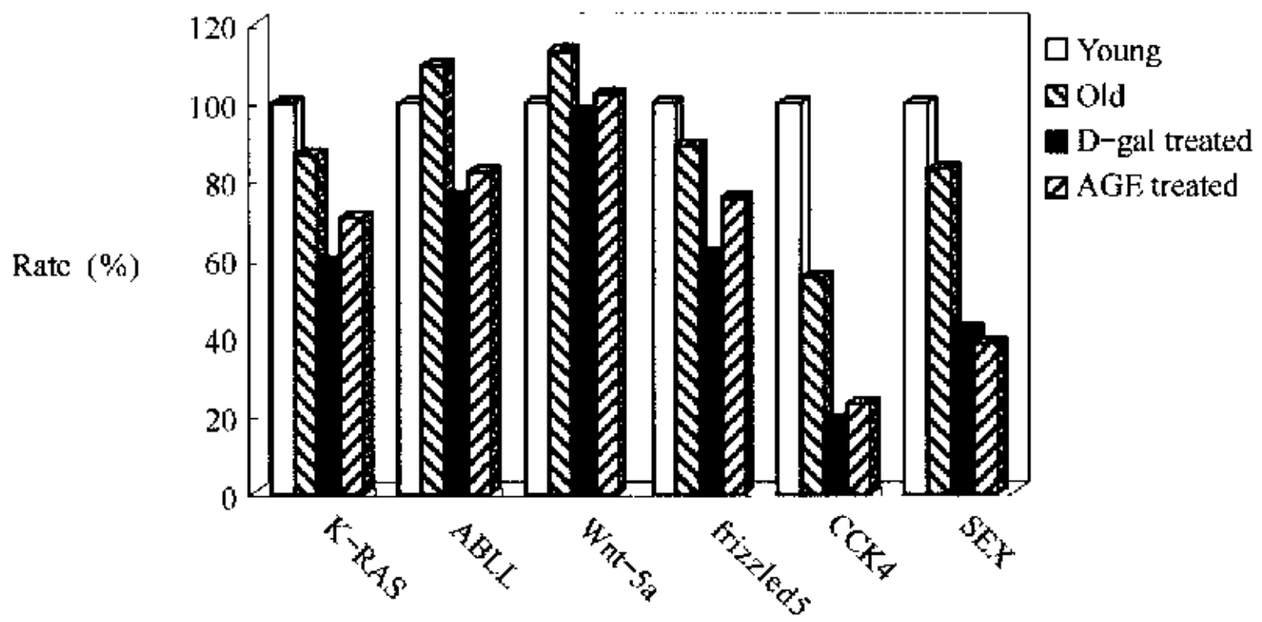

FIG. 1-3. Expression of oncogenes, cell fate/development regulators and receptors.

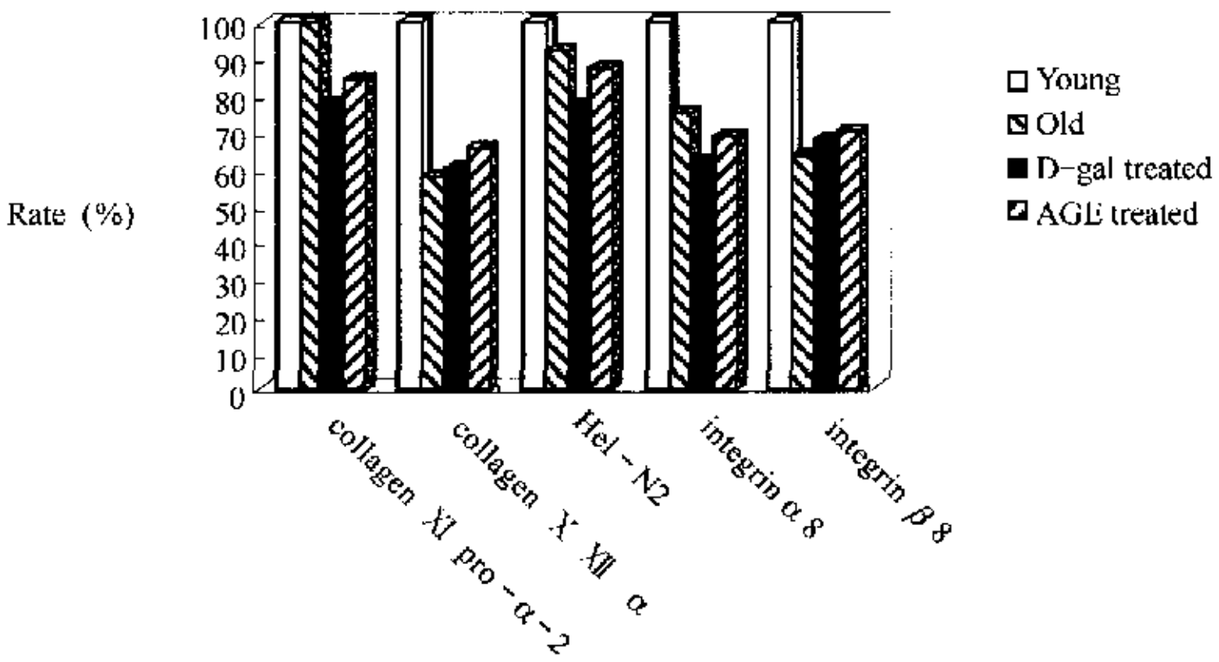

FIG. 1-4. Expression of genes involved in cell adhesion, motility and invasion.

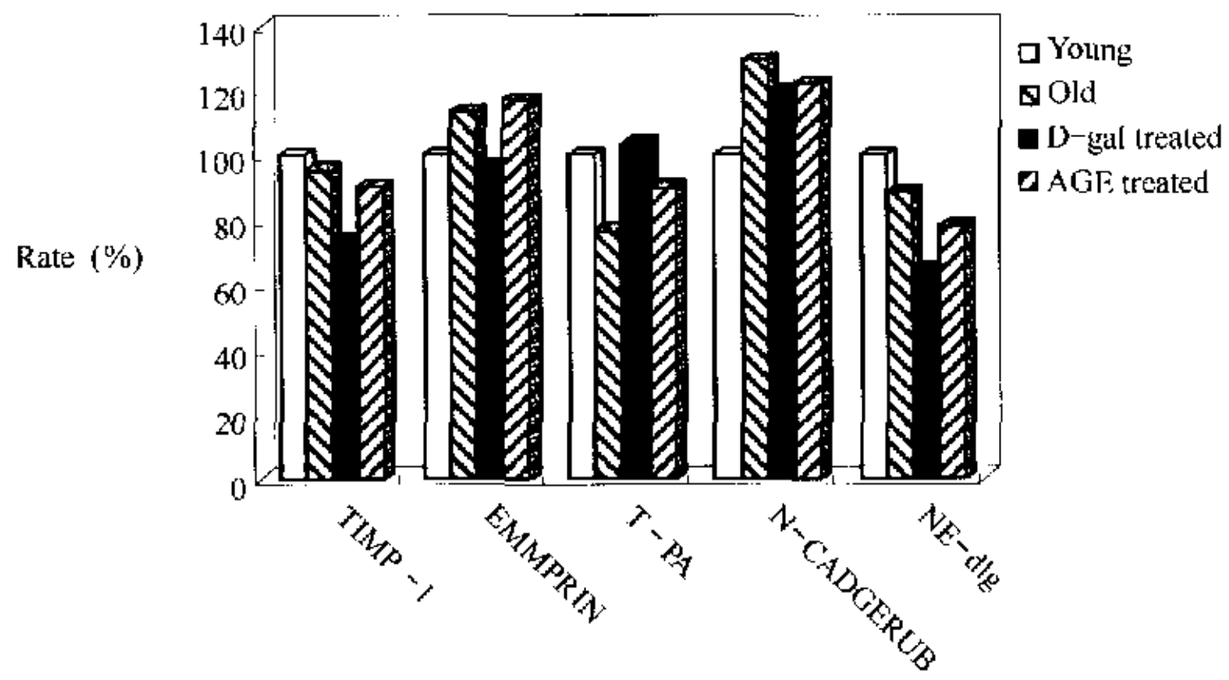

FIG. 1-5. Expression of genes involved in invasion regulators and cell-cell interactions.

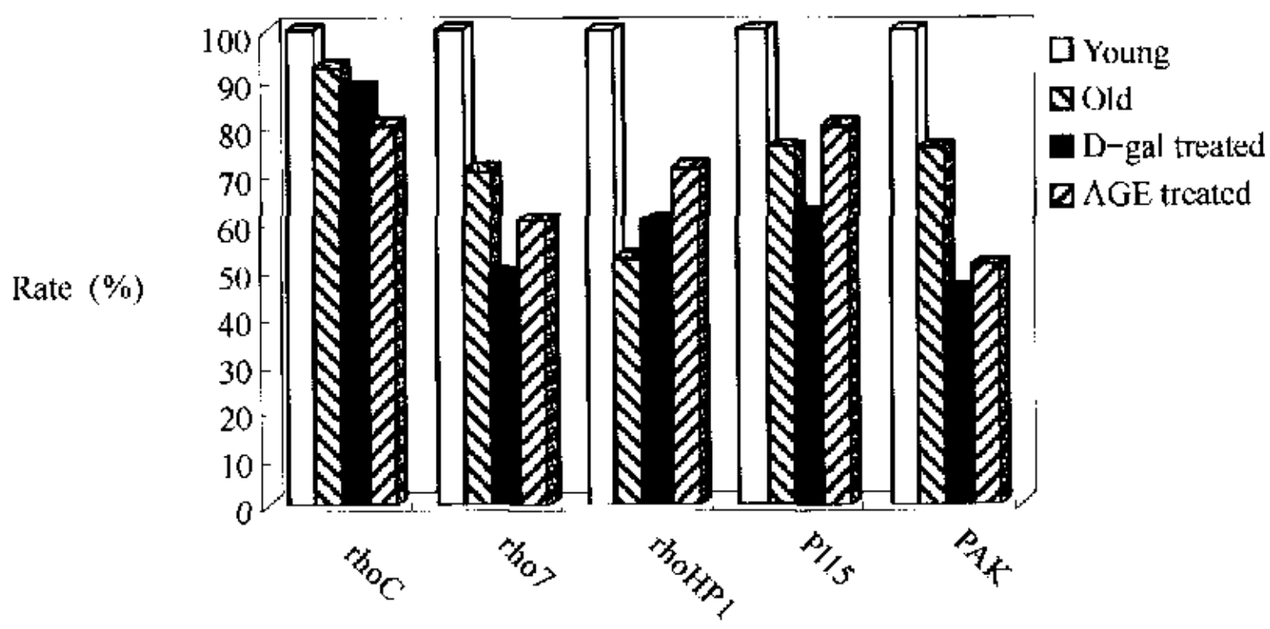

FIG. 1-6. Expression of rho family small GTPases and their regulators.

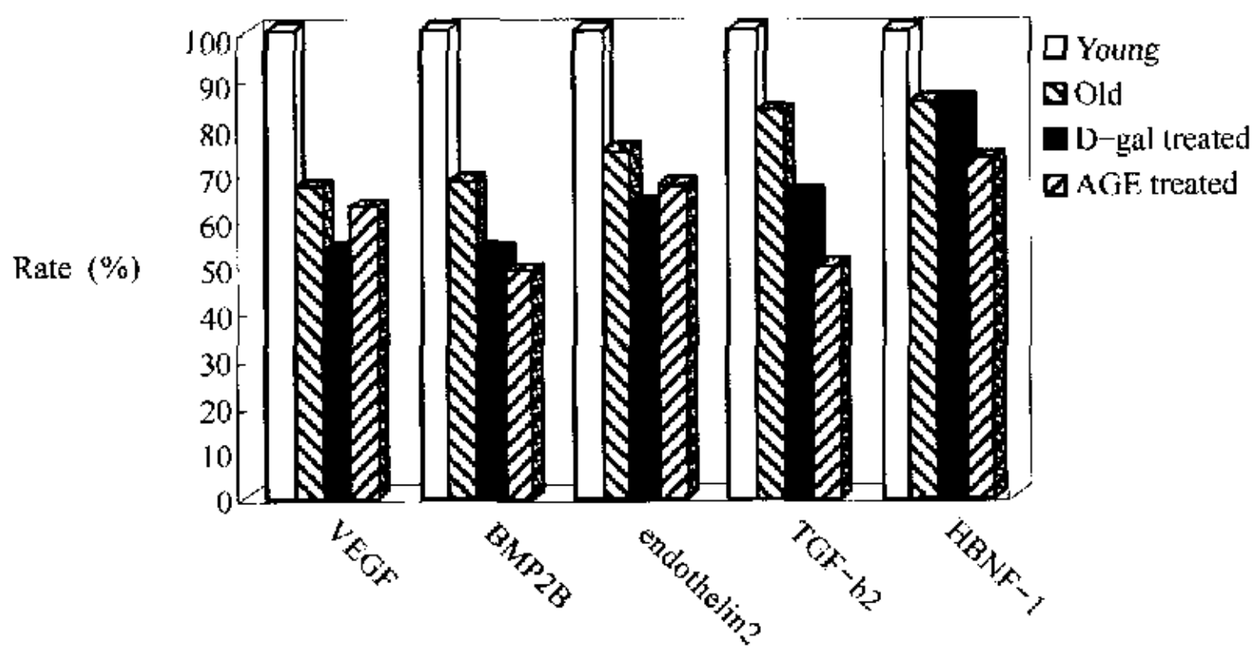

FIG. 1-7a. Expression of growth factors and cytokines.

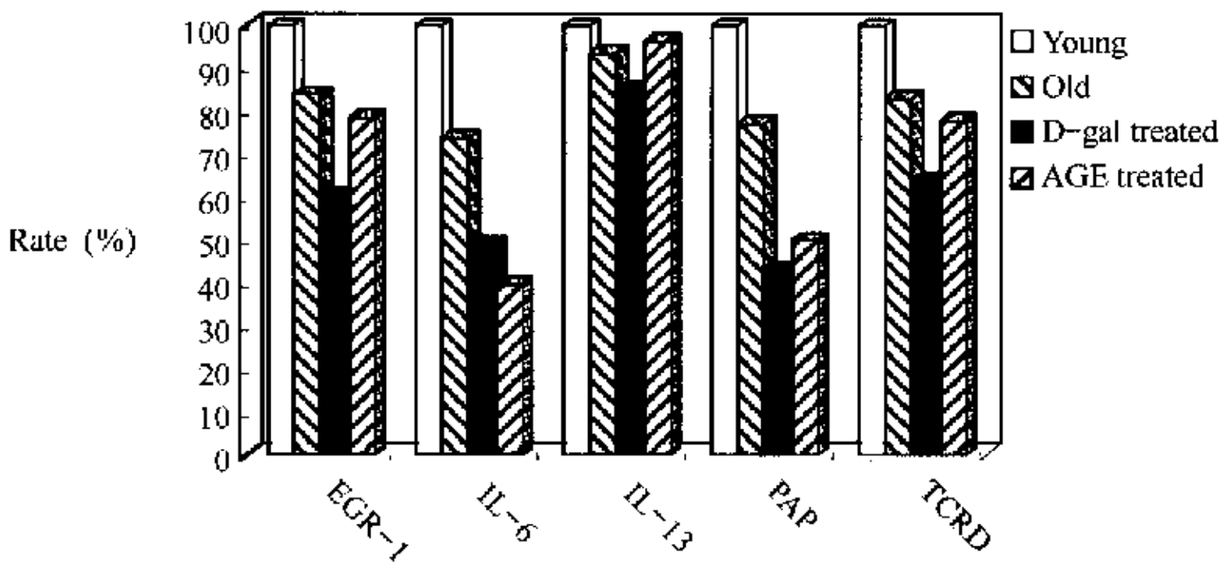

FIG. 1-7b. Expression of growth factors and cytokines.

FIG.1. Gene expression changes in the cerebrum from young, old, D-galactose treated and AGE treated female C57 BL/6J mice. Young: 5-month-old mice injected daily with 0.4 mL PBS; Old: 18-month-old mice injected daily with 0.5 mL PBS; D-galactose treated: 5-month-old mice injected daily with D-galactose (50 mg/kg); AGE treated: 5-month-old mice injected daily with D-galactose-modified AGE-lysine (50 mg/kg).

level superior to background. Compared with the young mice, the splenic lymphocytes from aged mice showed altered expression of 32 genes, among which 29 (34%) genes displayed an age-related decrease in expression, whereas TRIP, TRAF3 and LRP displayed an increase of expression levels with aging (Fig. 2).

DISCUSSION

Senescence is a progressive and irreversible physiological decline existing in most multicellular organisms, the postulated mechanisms of which include production of reactive oxygen species, which leads to oxidative damage to critical macromolecules, telomere shortening in replicative cells, instability of nuclear and mitochondrial genomes, epigenetic alterations leading to altered gene expression patterns, and nonenzymatic glycation of long-lived proteins[16]. The senescence-associated phenotypic changes are likely determined

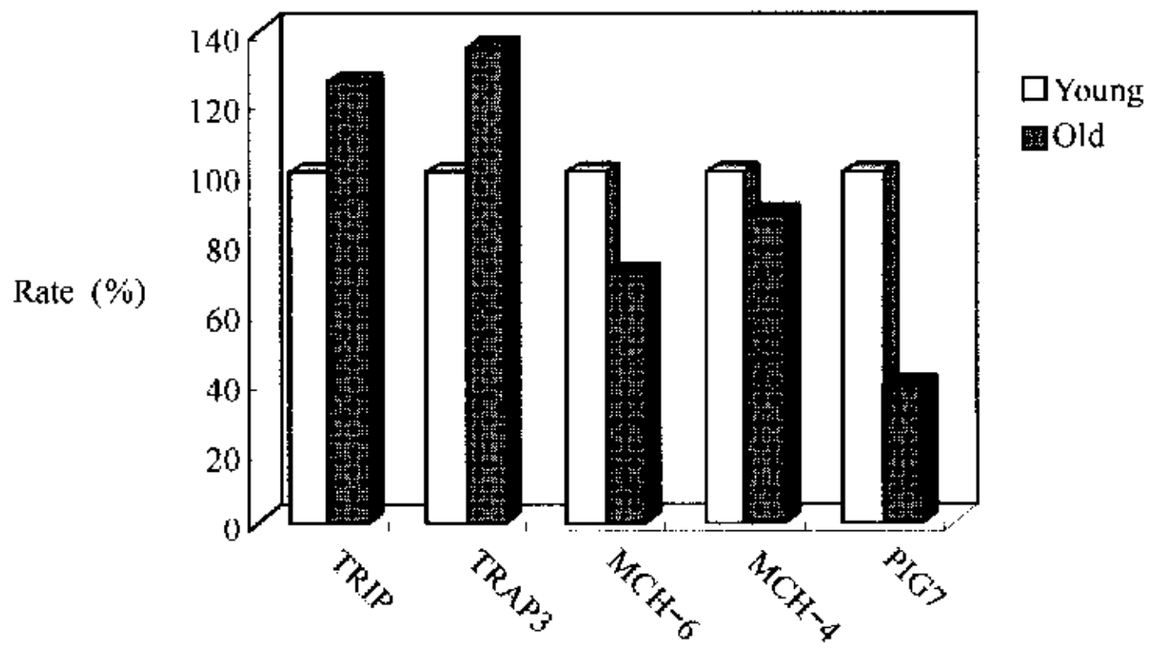

FIG. 2-1. Expression of apoptosis genes.

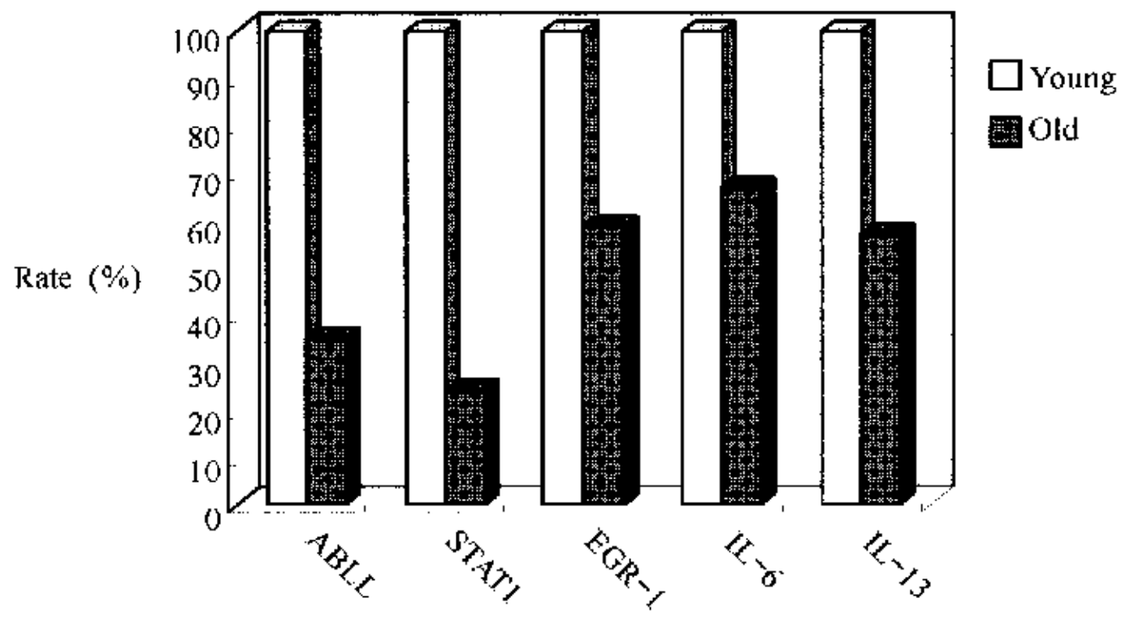

FIG. 2-2. Expression of oncogenes, growth factors and cytokines.

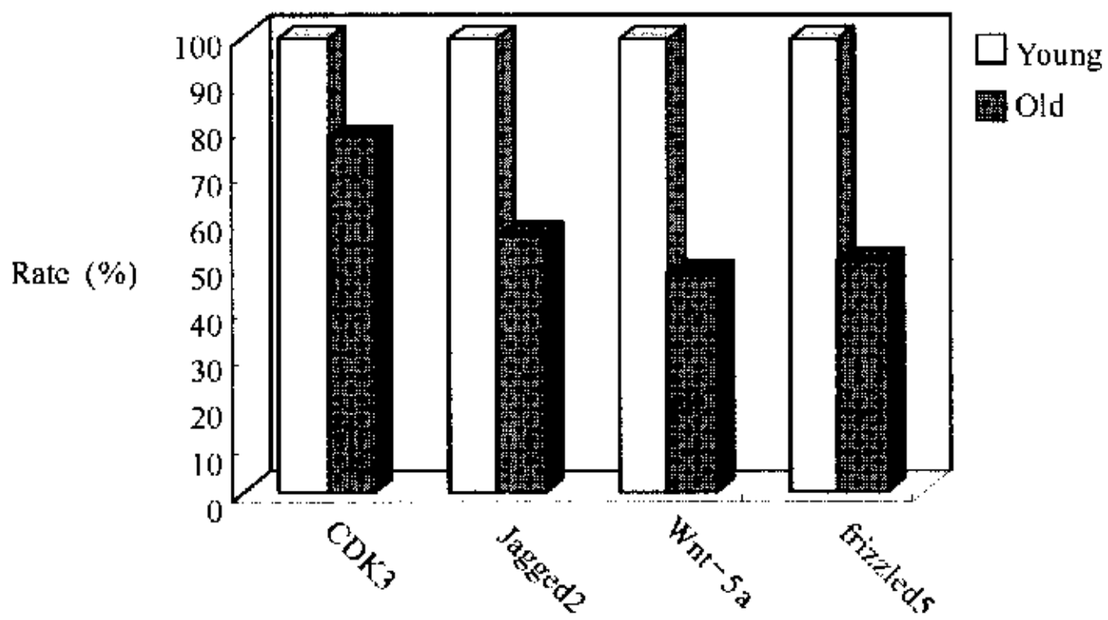

FIG. 2-3. Expression of cell cycle/growth regulators and cell fate/development regulators.

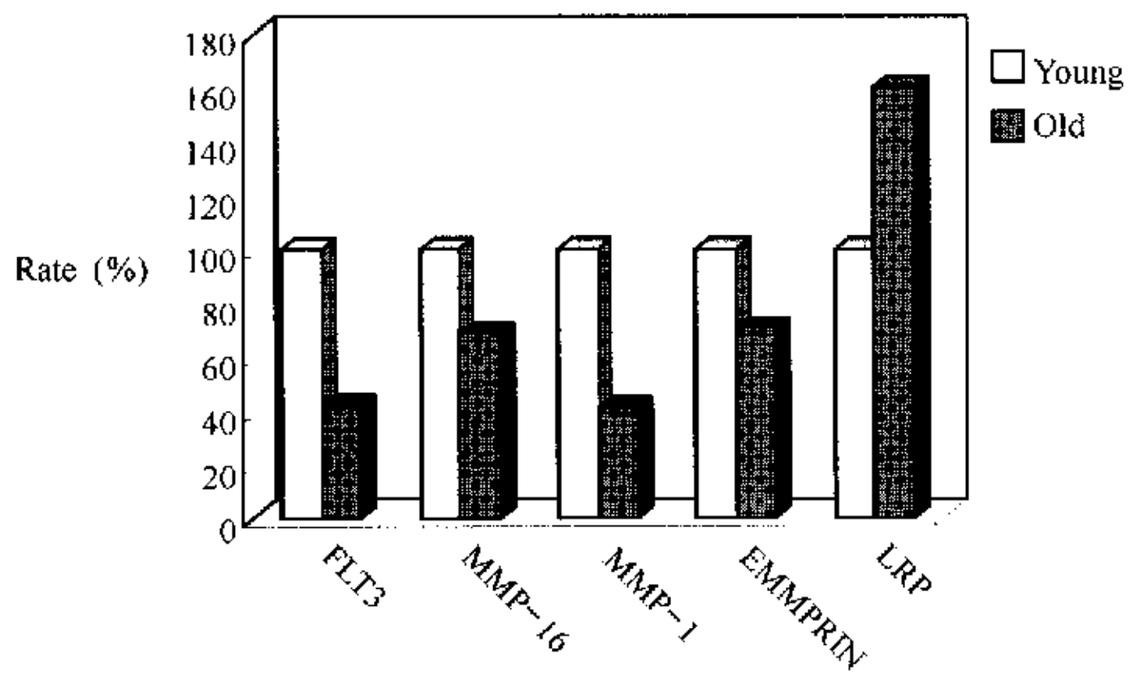

FIG. 2-4. Expression of angiogenesis regulators and invasion regulators.

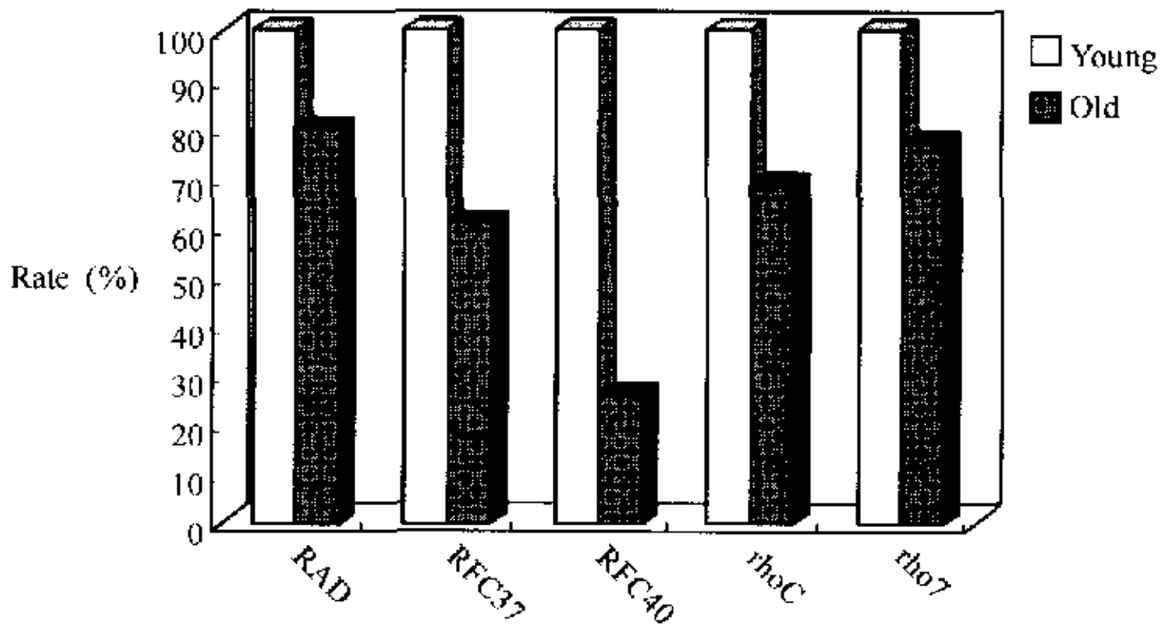

FIG. 2-5. Expression of genes involved in DNA damage response/repair/recombination and rho family small GTPases and their regulators.

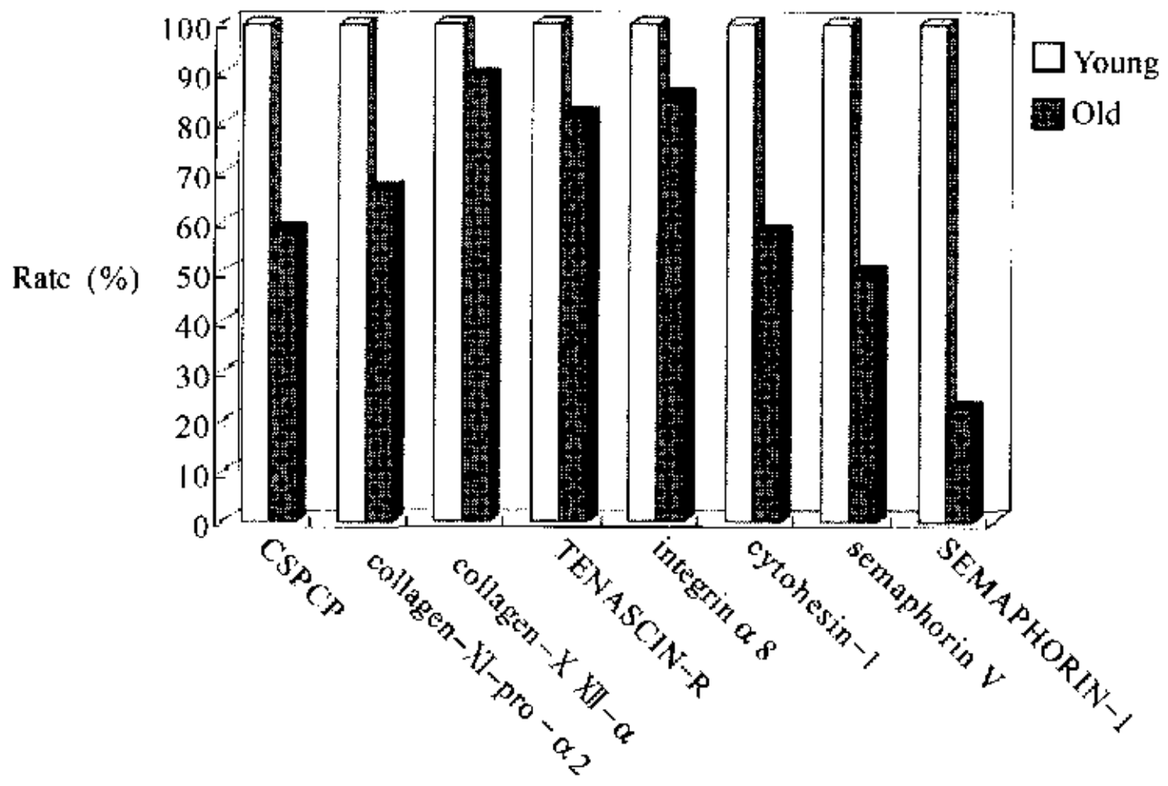

FIG. 2-6. Expression of genes involved in cell adhesion, motility and invasion.

FIG. 2. Gene expression changes in the splenic lymphocytes from young and old female C57 BL/6J mice. Young: 5-month-old mice injected daily with 0.4 mL PBS; Old: 18-month-old mice injected daily with 0.5 mL PBS.

by multiple genes whose products change in relative amounts and/or specific activities. Regulations at the transcriptional level are important to gene expression of eucaryote[7]. It is clear that transcriptional alterations of the aging process will be highly informative in understanding basic aspects of aging.

It has been proved that reducing sugars can also react with DNA as in the case of proteins to form AGE-DNA. Nucleic acids are long-lived molecules in resting cells, thus AGE-DNA could accumulate progressively with time. Such an accumulation may play a crucial role in aging-associated DNA alterations including DNA strand breaks, chromosomal alterations, a decline in DNA repair/ replication/ transcription and so on[8].

To examine the molecular events associated with AGE treatment in mammals, we compared cerebrum from old mice and young mice treated with PBS, D-galactose or AGE-

lysine. The results showed that changes in young mice treated with D-galactose or AGE-lysine were similar to those in old mice at the mRNA level, which might represent changes in gene expression, mRNA stability or turnover. An earlier report showed that AGE was a crucial mediator in the galactose-induced aging model, and advanced glycation might account, at least partially, for the mechanism of this aging model[9,10]. Nonenzymatic glycation may be associated with the genetic manipulation of the aging process. Postulated mechanisms of changes in gene expression of mice induced by nonenzymatic glycation include combined AGE and AGE receptor leading to altered gene expression patterns, AGE accumulation leading to genomic instability, and indirect effects of senescence-associated phenotypic changes induced by AGE on gene expression. The major classes of transcriptional alterations among the forty-three changed hybridization signals consist of apoptosis, invasion regulators, growth factors, and cytokines. Except for a few of the 43 genes associated with senescence which have been described previously, the effects of the rest on aging remain unknown. TNFR-associated factor 3 (TRAF3) is a candidate signaling molecule for the non-death-domain TNFR (tumor necrosis factor receptor) family members, which interacts with CD30, CD40, TNFR80, and LTβR, but not significantly with TNFR60 or Fas. TRAF3 is implicated in LTβR-mediated cell death response, but does not activate NF-κB[11]. TRAIL induces apoptosis through two closely related receptors, DR4 and DR5. The two receptors bind to the adaptor molecules FADD and TRADD, and can signal both death and gene transcription[12]. Colon carcinoma kinase 4 (CCK-4) is a member of the receptor tyrosine kinase family, which is involved in signal amplifying or modulating[13]. IL-6 is a pleiotropic cytokine. The binding of IL-6 to its receptor induces the activation of multiple signal transduction pathways such as JAK/STATs pathway, Ras/ERK pathway, and PI-3K/Akt pathway via gp130 tyrosine phosphorylation, and consequently IL-6 has complex biological functions[14].

In order to compare gene expression profile of splenic lymphocytes from young and old mice, we hybridized cDNAs to identical membrane arrays. We found some gene expression levels were down-regulated evidently. ABLL, encoded by a proto-oncogene, is a tyrosine kinase hypothesized to function in proliferation-stimulatory signaling pathways[15]. JAKs and STATs are central to the responses to the majority of cytokines and some growth factors. They transduce extracellular signals into nucleus resulting in transcriptional activation of target genes, and STAT1 has been found to exert proapoptotic effects in some cases[16]. Likely to be involved in slowed DNA replication with aging, expressions of RFC37 and RFC40 in old mice decreased markedly. Semaphorins are a large family of secreted and membrane-bound proteins that have multiple and diverse functions. Flk2-ligand, displayed on immature hematopoietic cells, is a angiogenesis regulator. Decreased expressions of semaphorin-1 and Flk2-ligand may be the responses of cells to aging. Low density lipoprotein receptor-related protein (LRP) is an endocytic receptor, and delivers cholesterol-containing lipoproteins and other ligands to acidic compartments in cells for further metabolism. Ligands of LRP include proteins that are related to lipid metabolism, proteinase regulation, blood coagulation/fibrinolysis cascades, and several membrane proteins, such as urokinase plasminogen activator receptor and β-amyloid precursor protein[17]. LRP may be both an endocytic and a signaling receptor, which may be of relevance to the role of LRP and its ligands in aging. Senescence is a very complex process, which undergoes changes in various tissues and organs. The cells of cerebrum and spleen may show similar tendency of change of signal transduction and physiological effect in aging process. Compared with the young mice, 18 genes altered at mRNA levels in the aged splenic lymphocytes and cerebrum were shared; furthermore 11 (61%) changes were consistent. The data presented here provide some analysis of the molecular events associated with AGE treatment in mammals.

These transcriptional changes may be closely related to aging. More evidences are needed to confirm these results. We are undertaking a further research to determine which alterations are primary causal factors in aging process in order to get insights into aging mechanisms.

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