Mechanisms of Ageing and Development 133 (2012) 467–478

Contents lists available at SciVerse ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Meta-analysis of gene expression in the mouse liver reveals biomarkers associated with inflammation increased early during aging☆

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ARTICLE INFO

Article history:
Received 1 December 2011
Received in revised form 23 May 2012
Accepted 29 May 2012
Available online 13 June 2012

Keywords:
Aging
Longevity
Liver
Meta-analysis
Microarrays
Xenobiotic metabolism
Inflammation

ABSTRACT

Aging is associated with a loss of cellular homeostasis, a decline in physiological function and an increase in various pathologies. Employing a meta-analysis, hepatic gene expression profiles from four independent mouse aging studies were interrogated. There was little overlap in the number of genes or canonical pathways perturbed, suggesting that independent study-specific factors may play a significant role in determining age-dependent gene expression. However, 43 genes were consistently altered during aging in three or four of these studies, including those that (1) exhibited progressively increased expression starting from 12 months of age, (2) exhibited similar expression changes in models of progeria at young ages and dampened or no changes in old longevity mouse models, (3) were associated with inflammatory tertiary lymphoid neogenesis (TLN) associated with formation of ectopic lymphoid structures observed in chronically inflamed tissues, and (4) overlapped with genes perturbed by aging in brain, muscle, and lung. Surprisingly, around half of the genes altered by aging in wild-type mice exhibited similar expression changes in adult long-lived mice compared to wild-type controls, including those associated with intermediary metabolism and feminization of the male-dependent gene expression pattern. Genes unique to aging in wild-type mice included those linked to TLN.

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1. Introduction

Developed countries have observed an astonishing increase in human life expectancy over the last two centuries, with roughly 30 years being added to average life expectancy from birth since the start of the 20th century (Christensen et al., 2009). For example, while more than 12% of the population in the United States is currently over the age of 65, this will increase to nearly 20% by 2030 (He et al., 2005). These changing demographics will undoubtedly have significant implications for society as a whole, because aging is inevitably linked to physiological decline, loss of independence, and increases in a range of pathologies with subsequent decreases in the quality of life (Vaupel, 2010).

Although advances in medicine and better nutrition have contributed to an increase in longevity, the growing population of older Americans has the potential for greater susceptibility to adverse health effects from environmental pollutants due in part to changes in protective mechanisms that respond to stressors. For example, several studies have found older adults to be especially sensitive to air pollutants (Fischer et al., 2003; Gouveia and Fletcher, 2000). Increased sensitivity may be due to remnant effects from past exposures as well as weakened immune responses (Sandstrom et al., 2003). The aging population may

☆ The information in this document has been funded by the U.S. Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Abbreviations: Ct, cycle threshold; PCR, polymerase chain reaction; XME, xenobiotic metabolizing enzyme.

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0047-6374/$ – see front matter. Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.mad.2012.05.006

also be more susceptible to environmental exposures because of a decreased ability to detoxify and transport chemicals out of the body. A number of xenobiotic metabolizing enzymes were shown to change with age in rats (Lee et al., 2008; Mori et al., 2007) and mice (Lee et al., 2011) and these changes could affect the ability to detoxify environmental chemicals as well as drugs. Additionally, liver size and hepatic blood flow decreases with age, despite hepatocyte volume remaining static, which may affect the elimination of high-clearance drugs (Klotz, 2009).

The use of microarray studies has been critical in helping to identify potential candidate mechanisms underlying the aging process (Lee et al., 2000). Microarray studies can be integrated by either re-analyzing primary data from multiple studies that have been computationally normalized, or, using less useful techniques, by comparing gene lists (Cahan et al., 2007). Such meta-analysis can increase the statistical power to detect small, but consistent effects that might be false negatives in the individual analyses (Choi et al., 2003). Using multiple datasets in a meta-analysis can be a powerful tool to interrogate existing databases for candidate biomarkers and biological pathways useful for diagnosis and biomonitoring (Hong and Breitling, 2008).

Meta-analysis of microarray datasets has provided insights into the mechanisms of aging. By comparing gene expression patterns across species of low complexity, McCarroll et al. found a transcriptional profile indicative of aging (McCarroll et al., 2004). The nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* implement a shared adult-onset expression program of genes involved in mitochondrial metabolism, DNA repair, catabolism, peptidolysis and cellular transport, as well as conserved transcriptional signatures in larval development, embryogenesis, gametogenesis and mRNA degradation (McCarroll et al., 2004). A meta-analysis of microarray studies identified an expression signature that is common to the aging transcriptomes of mouse, human and rat, and conserved within species, but also within organs across species (Wennmalm et al., 2005). Further, similarities between the expression signatures of cellular senescence and aging were found in mice, but not in humans (Wennmalm et al., 2005). de Magalhaes et al. used 27 datasets from mice, rats and humans in their meta-analysis of age-related gene expression profiles (de Magalhaes et al., 2009). They observed several common signatures of aging, including 56 genes consistently overexpressed with age, the most significant of which was apolipoprotein D (*APOD*), and 17 genes consistently underexpressed with age (de Magalhaes et al., 2009). With aging, an increased expression of inflammation, immune response and lysosome-associated genes was observed, in parallel with decreased expression of collagen genes and genes associated with energy metabolism (de Magalhaes et al., 2009). McElwee et al. (2007) applied a multi-level cross-species comparative analysis to identify gene expression changes accompanying increased longevity in mutant nematodes, fruitflies and mice with reduced insulin/insulin-like growth factor (IGF)-I signaling (IIS), and found many gene families linked to xenobiotic metabolism showing conserved up-regulation, suggesting detoxification to be a plausible mechanism of longevity assurance.

A biomarker is an objectively measured indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention (Butler et al., 2004). Biomarkers can be used to understand physiological processes that change with age, diseases whose onset appear linked to age, and the aging process itself; however, there is no agreed upon set of biomarkers of aging (Crimmins et al., 2008). Biomarkers related to aging have been identified from large-scale community and population studies, such as the Framingham study and the National Health and Nutrition Examination Survey (NHANES) (Crimmins et al., 2008). Although a number of studies have been carried out in which altered gene expression in mouse liver have been identified, no comprehensive comparative analysis of these datasets have been undertaken to identify reliable biomarkers of aging in the liver.

To gain insights into the aging process, to identify and characterize biomarkers of aging, and to better understand the relationships between groups of genes regulated by both longevity and aging, we performed a meta-analysis on whole-genome hepatic gene expression changes in mice and compared the results to transcriptional profiles in other mouse aging studies. Surprisingly, only a small fraction of the total number of genes altered by aging was common across all four independent studies. However, the genes that were consistently altered in most of these studies were associated with inflammation commonly observed in aged tissues. This core set of genes showed altered expression during mid-life, thus providing opportunities for their use in monitoring interventions proposed to alter aging.

2. Materials and methods

2.1. Animals and study design

Male C57BL/6J mice, at approximately 6, 12, 18 and 24 months of age, were obtained from Charles River Laboratory (Raleigh, NC) and acclimated for 1 week. Mice were housed in polycarbonate cages on Alpha Dry bedding with a 12-h light/dark cycle. Room temperature was 21 ± 2 °C with a relative humidity of 50%. The basal diet, Ralston Purina 5001, and water were provided *ad libitum*. Animals at 6, 12, 18 and 24 months of age were sacrificed in the morning using CO_2 asphyxiation. Livers were removed, weighed, cubed and stored at −80 °C until RNA isolation. All aspects of these studies were conducted in compliance with the guidelines of the AAALAC-International and approved by the US EPA/NHEERL Institutional Animal Care and Use Committee (IACUC).

2.2. RNA isolation

Total RNA was isolated from mouse livers according to the TriReagent procedure (Molecular Research Center, Cincinnati, OH) and purified using the Qiagen RNeasy mini RNA cleanup protocol (Qiagen, Valencia, CA). The integrity of each RNA sample was determined using an Agilent 2100 Bioanalyzer (Agilent, Foster City, CA). RNA quantity was determined using a Nanodrop® ND-1000 (Thermo Fisher Scientific, Wilmington, DE).

2.3. Microarray hybridizations

Liver gene expression analysis was performed according to the recommended protocol using Affymetrix Mouse Genome 430 2.0 GeneChips® containing probes for over 30,000 genes. Total RNA (5 μg per sample) was labeled using the Affymetrix® One-Cycle cDNA synthesis protocol and hybridized to arrays as described by the manufacturer (Affymetrix®, Santa Clara, CA). The cRNA hybridization cocktail was incubated overnight at 45 °C while rotating in a hybridization oven. After 16 h of hybridization, the cocktail was removed and the arrays were washed and stained in an Affymetrix GeneChip® fluidics station 450 according to the Affymetrix-recommended protocol. Arrays were scanned on an Affymetrix GeneChip® scanner. Four mice per age group were examined and cRNAs from individual mouse livers were hybridized to individual chips.

2.4. Analyses of microarray data

All the Affymetrix.cel files were first analyzed by Bioconductor SimpleAffy to assess data quality (Wilson and Miller, 2005). All .cel files passed this QC step. Data (.cel files) were background corrected and statistically filtered using Rosetta Resolver version 7.1 software (Rosetta Inpharmatics, Kirkland, WA). The background correction was done by Resolver's specific data processing pipeline (Affymetrix Rosetta-Intensity Profile Builder). Statistically significant genes were identified using one-way ANOVA with a false discovery rate (Benjamini–Hochberg test) of $\leq$0.05, followed by a *post hoc* test (Scheffé) for significance.

2.5. Meta-analysis

We compared our results to a number of other studies which examined gene expression in the livers of aged mice (Table 1). Aging studies used in comparisons to our results included the wild-type and long-lived counterparts of Ames (Amador-Noguez et al., 2004; Boylston et al., 2006, data not shown), Little (Amador-Noguez et al., 2004) and Snell dwarf (Boylston et al., 2004) mice, as well as progeroid mice (Schumacher et al., 2008), a mouse model of accelerated aging (SAM-P8) (Vila et al., 2008), and calorically restricted C57BL/6J mice (Fu et al., 2006; Selman et al., 2006). We also compared our results to the Atlas of Gene Expression in Mouse Aging