Changes in Gene Expression Related to the Decline of the Aging Immune System

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Abstract

In this study, the gene expression levels between 6 month old mice and 26 month old mice were compared. The purpose of this research was to identify genes that are differentially regulated due to aging. The results showed 148 significantly differentiated genes in the analysis of a total of 44,170 genes with 16 genes found to be related to immunity and inflammation.

Introduction

As we age, our body's fragile balance of gene expression in cells gradually begins to go awry due to many factors such as genetics and the environment. The body's declining state contributes to the development of chronic diseases, such as infections, cardiovascular and neurological diseases. Researchers have coined a key-term, "inflamm-aging", to describe the increase in inflammation linked to diseases and illness due to aging. (Franceschi et al., 2000)

"Inflamm-aging" is characterized as the development of a low-level inflammatory status caused by up-regulation of pro-inflammatory cytokines during the aging process. There is also a decline in the adaptive immune system and over-activity in the innate immune system, which is the first line of defense against infection involving macrophages and neutrophils. The bone marrow produces all the cells of the immune system; therefore, we hypothesized changes in gene expression in bone marrow cells occur during the process of aging. To test this hypothesis, we compared expression of 44,170 genes in bone marrow cells from 6 month old versus 26 month old mice.

Materials and Methods

cDNA synthesis of pooled RNA samples

Twenty-four RNA samples isolated from the bone marrow cells of young and old C57Bl6 mice were pooled into eight groups of three samples (four groups of 6 month and four of 26 month old mice) (Table 1).

Samples	Pool #	Age (months)
545, 546B, 547	1	3
548B, 549, 550B	2	3
535, 536B, 537	3	3
538B, 539, 540B	4	3
559, 560B, 561	5	26
562B, 563, 564B	6	26
553, 554B, 555	7	26
556B, 557, 558B	8	26

Table 1. Individual RNA samples were pooled into 8 different groups.

The pooled RNA (1μg/μL) was synthesized into cDNA using either the cDNA Synthesis System (Roche NimbleGen) or the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen). The cDNA was purified using a DNA clean & Concentrator-5 Kit (ZymoGenetics). Confirmation of the cDNA integrity was performed using electrophoresis of 100 ng of each cDNA through a 1% agarose gel followed by staining with ethidium bromide.

Labeling of cDNA (Invitrogen and Nimblegen)

The cDNA was labeled with Cy3 Random Nonamers and the Klenow Fragment using the NimbleGen One-Color DNA Labeling Kit as described by the manufacturer (Roche). Quality of the labeled cDNA was verified by electrophoresis through a 1% agarose gel to observe the intensity of the Cy3 fluorescence label.

Hybridization and microarray analysis

The labeled cDNA was sent to the Center for Genome Research and Biocomputing for hybridization onto a 12x8 microarray chip (Roche) and processed. Results were analyzed using BRB ArrayTools (National Cancer Institute).

Results

We compared young mice (6 months) versus old mice (26 months) and generated a list of 148 genes that statistically and significantly differed between the two populations. A majority of the genes encoded immunoglobulin proteins. Genes expressed by B-cells, T-cells, macrophages, or neutrophils were also identified.

Some of the genes related to immunity and inflammation were significantly up-regulated in old mice versus young mice. Epcam (5.5-fold) encodes a cell surface marker of epithelial cells that is also found on erythroid progenitor cells (Lammers et al., 2002). CD5L (2.1-fold) regulates of the immune system and inhibits apoptosis. Prdm1 (2.3-fold), encodes a pre-lymphocyte-induced maturation protein and Sdc1 (1.9-fold) encodes an integral membrane protein involved in cell proliferation and cell migration.

Some genes that were significantly down-regulated in old mice were Cd79a (-2-fold), a B-cell antigen receptor complex-associated protein alpha chain; Epor (-2-fold) that encodes an erythropoietin receptor important for erythroid cell survival; Sparc (-2-fold), which appears to regulate cell growth through interactions with the extracellular matrix and cytokines; Fam129c (-2-fold) encodes a novel B-cell protein important for immune response; Cpm (-2-fold), carboxypeptidase M, is associated with monocyte to macrophage differentiation and Vpreb3 (-3-fold) encodes pre-B lymphocyte gene 3.

Discussion

We have identified many genes of interest that were altered during aging of mice. To further investigate these genes and their impact on aging, we will confirm their differences in expression with qRT-PCR to verify the results from the microarray data. Furthermore, significant work will need to be performed to determine if the altered expression of these genes provides mechanistic insight into the decline of the aging immune system.

References

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