Short Communication

Journal of Biomedical Science

J Biomed Sci 2004;11:528–530
DOI: 10.1159/000077903

Received: July 14, 2003
Accepted: January 16, 2004

Decreased Expression of the Pancreatic Secretory Trypsin Inhibitor II Gene during Aging of the Rat Liver

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Key Words

Aging · Differential display · Gene expression · Liver · Pancreatic secretory trypsin inhibitor II · Trypsin

Abstract

The genetic constitution and differential gene expression of an organism play important roles in controlling the species-specific rate of aging and the maximum life span potential. We utilized a differential-display polymerase chain reaction technique to identify the age-dependent expression of genes in the rat liver. We demonstrate in this report, for the first time, that expression of the pancreatic secretory trypsin inhibitor II (PSTI-II) gene declines drastically during aging. We confirmed this decrease by Northern blot analysis. Low PSTI-II levels in aged animals might result in a lack of protection from prematurely activated trypsin-like proteases, which would thus enhance inflammation.

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Supported by funds from the Department of Biotechnology and by Research Fellowships (to H.P. and G.Z.) from the Council of Scientific and Industrial Research (Government of India).

Introduction

Aging processes are time-dependent changes with deteriorating functions that lead to progressive loss of the ability of an organism to withstand both internal and external stresses, resulting in failure of cellular homeostasis. Various reports have suggested that differential gene expression occurs during the aging process in all mammalian cells [7, 8, 12, 13]. The differential-display polymerase chain reaction (DD-PCR) technique has been widely used to monitor differential expression of genes [6]. The DD-PCR has revealed that only a small number (~ 1%) of genes are differentially expressed during aging in particular tissues. Increased and decreased expression of genes during aging has been reported in the rat brain, liver, and heart [1, 4, 10], as well as in aged cultured fibroblasts [8]. Calorie restriction, the only regimen known to delay the aging process, has also been reported to delay the onset of the expression of certain deleterious genes [5]. In an attempt to identify age-dependent genes in the rat liver, we carried out DD-PCR experiments in the liver RNA of adult and old rats. Using a combination of 3 one-base anchored oligo-dT primers and 8 arbitrary primers, we have identified 15 cDNA bands whose expressions are altered during aging. In this report, we describe two of these cDNA bands and demonstrate the decreased expression of the pancreatic secretory trypsin inhibitor II gene in the liver of old rats.

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1021–7770/04/0114–0528$21.00/0
Accessible online at:
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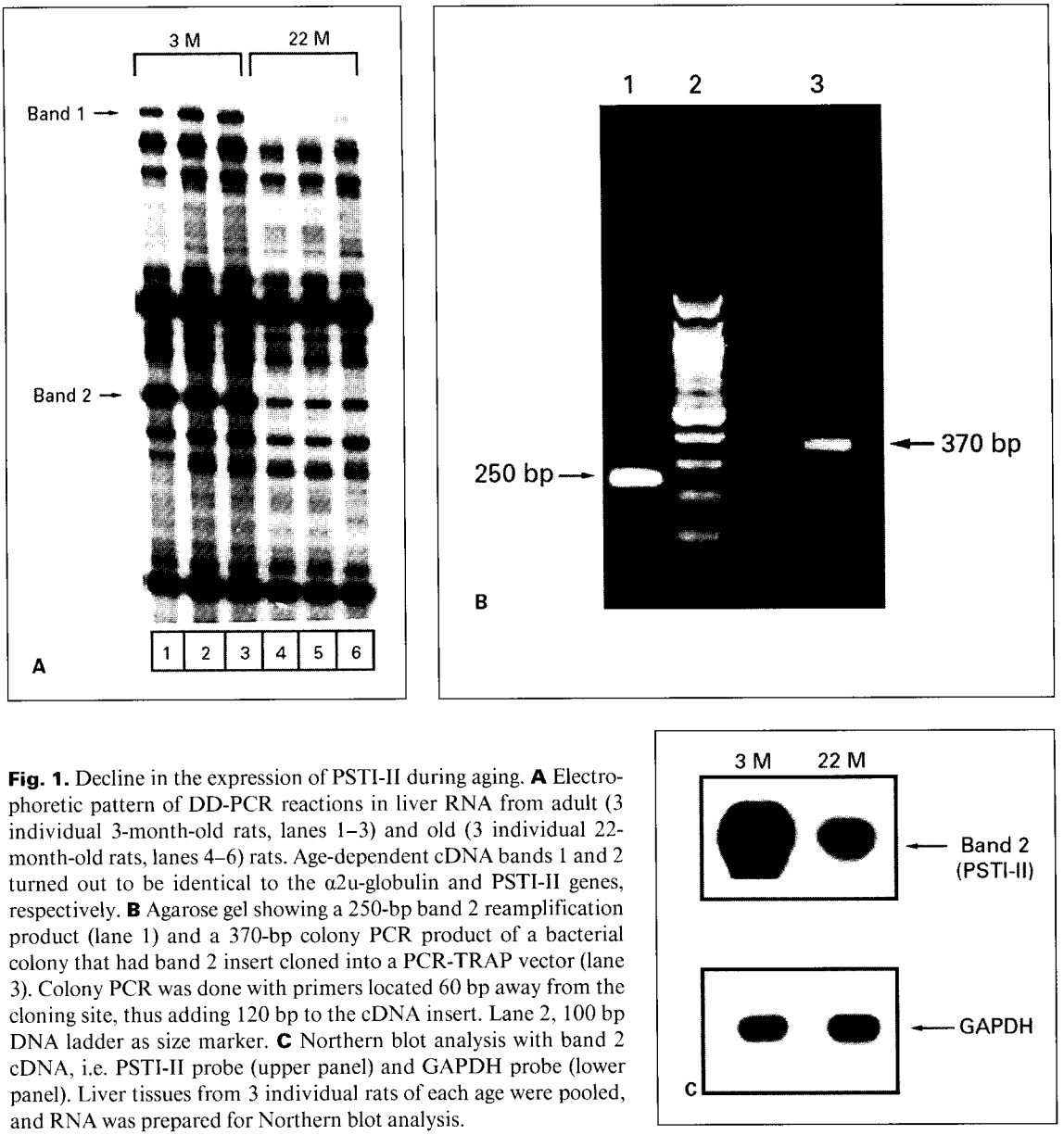

Fig. 1. Decline in the expression of PSTI-II during aging. **A** Electrophoretic pattern of DD-PCR reactions in liver RNA from adult (3 individual 3-month-old rats, lanes 1–3) and old (3 individual 22-month-old rats, lanes 4–6) rats. Age-dependent cDNA bands 1 and 2 turned out to be identical to the α2u-globulin and PSTI-II genes, respectively. **B** Agarose gel showing a 250-bp band 2 reamplification product (lane 1) and a 370-bp colony PCR product of a bacterial colony that had band 2 insert cloned into a PCR-TRAP vector (lane 3). Colony PCR was done with primers located 60 bp away from the cloning site, thus adding 120 bp to the cDNA insert. Lane 2, 100 bp DNA ladder as size marker. **C** Northern blot analysis with band 2 cDNA, i.e. PSTI-II probe (upper panel) and GAPDH probe (lower panel). Liver tissues from 3 individual rats of each age were pooled, and RNA was prepared for Northern blot analysis.

Materials and Methods

Total RNA was prepared from the liver of 3 individual adult (aged 3 months) and 3 individual old (aged 22 months) Fisher 344 rats using the Trizol reagent (Invitrogen, Grand Island, N.Y., USA). All animal care and procedures were approved by our Institutional Animal Ethics Committee. All RNA samples were treated with DNase I in the presence of RNase inhibitors to remove any contaminating genomic DNA. RNA (200 ng) was reverse transcribed in 20 μl reaction buffer containing 400 μ*M* dNTP, 4 μ*M* of an anchored oligo dT primer (H-T11A, 5′-AAGCTTTTTTTTTTTA-3′; GenHunter, Nashville, Tenn., USA) and 200 units of MMLV reverse transcriptase. The reverse transcribed product (cDNAs) was then subjected to the PCR reaction. The reaction contained 2 μl of cDNA, 40 μ*M* dNTP, 3 μCi of ^{32}P-αdCTP (4,000 Ci/ mmol), 4 μ*M* of the H-T11A primer, 4 μ*M* of the arbitrary primer (H-AP1, 5′-AAGCTT-GATTGCC-3′; GenHunter) and 1 unit of Taq polymerase (Promega, Madison, Wisc., USA). PCR conditions were as follows: denaturation at 94 °C for 30 s, annealing at 40 °C for 2 min, and extension at 72 °C for 30 s. After 40 cycles of amplification, additional extension was performed for 5 min. Samples were denatured and electrophoresed in a 6% polyacrylamide-urea gel. After autoradiography, the differentially expressed cDNA bands were excised from the gel, and DNA was isolated. Eluted DNA was reamplified using the same combination of anchored and arbitrary primers as described above except that dNTP concentration was 800 μ*M* and no radioisotope

was used. Reamplified cDNA was checked on an agarose gel and was cloned into the PCR-TRAP cloning vector (GenHunter). Bacterial colonies were checked for the cloned insert by PCR using vector primer flanking the cloning site. Plasmid DNA was prepared and the insert was sequenced using vector primers from both directions with a Thermo Sequenase cycle sequencing kit (United States Biochemicals, Cleveland, Ohio, USA). The GenBank database was searched for any homology with the nucleotide sequence. The differential expression was confirmed by Northern blotting [11]. Total RNA samples (20 μg) were fractionated on a 1% agarose/formaldehyde denaturing gel and transferred to Hybond N+ nylon membrane (Amersham, Amersham, UK) by vacuum transfer. After crosslinking and prehybidization, membranes were hybridized with a ^{32}P-labeled DNA probe generated by nick-translation of reamplified DNA. Membranes were washed and exposed to X-ray film for autoradiography. A GAPDH (glyceraldehyde-3-phosphate dehydrogenase) probe was used as a control for RNA loading.

Results

Figure 1A shows the electrophoretic pattern of the DD-PCR carried out with liver RNA of 3 adult rats (aged 3 months) and 3 old rats (aged 22 months). The expression of two bands (band 1, 600 bp; band 2, 250 bp) shows significant decrease in their expression in old rats. Cloning and sequencing of band 1 revealed that it is identical to that of the α_2u-globulin gene, whose expression has already been reported to decline during aging in the liver [9], indicating that our DD-PCR result is authentic. DNA band 2 was reamplified (250 bp, fig. 1B, lane 1), and cloned into a PCR-TRAP vector, and bacterial colonies were checked for insertions by PCR primers located 60 bp away from the cloning site. As expected, a 370-bp band was observed after PCR (fig. 1B, lane 3). A nucleotide sequence search for band 2 in the GenBank database revealed that it is identical to that of rat pancreatic secretory trypsin inhibitor II (PSTI-II) mRNA (GenBank accession No. 23097351).

Discussion

In the rat, two forms of the pancreatic seretory trypsin inhibitor, PSTI-I and PSTI-II, are secreted into pancreatic juice, and they play a role in the protection of the pancreas from premature activation of protease-rich pancreatic juice. It has been demonstrated that PSTI-I is expressed in the pancreas, whereas PSTI-II is expressed in the pancreas as well as in the liver [2]. This is the first report to demonstrate a dramatic decline in the expression of the PSTI-II gene in rat liver by the DD-PCR. A decrease in PSTI-II gene expression was also confirmed by Northern blot analysis (fig. 1C). The human PSTI gene has been shown to be overexpressed in the liver of patients with adult-onset type II citrullinemia (characterized by a deficiency in argininosuccinate synthetase) [3]. PSTI may have a general inhibitory function against trypsin-like protease release in tissue injury, instead of being a purely local trypsin inhibitor. Lower PSTI-II levels in old tissue might result in a lack of protection from prematurely activated trypsin-like proteases and may enhance inflammation. Further studies are needed to understand the exact role of PSTI-II in the liver during aging.

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