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Differential Expression of Immunoglobulin Kappa Chain Constant Region in Human Abdominal Aortic Aneurysm

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Background. **A number of the research into the pathogenesis of the abdominal aortic aneurysm (AAA) have focused on the alteration of gene expression. The current technique for elucidating alterations of gene expression has a setback in that many artifact complementary DNA (cDNA) products present abnormal polymerase chain reaction (PCR) amplification. Our study was designed to identify differentially expressed genes in AAA using the annealing control primer (ACP) system, which was recently developed to identify only authentic genes.**

Materials and methods. **The tissues of the human abdominal aorta were obtained from the patients of AAA and aortic occlusive disease (AOD), and normal abdominal aorta (NA) from brain death donors. Total RNAs were isolated from three groups of human abdominal aorta (10 AAA, five NA, three AOD) and then reverse transcribed into complementary DNA (cDNA). The ACP method was done to screen the difference in the expression pattern of the mRNA (mRNA).**

Results. **One differentially expressed cDNA band was detected in AAA but not in NA and AOD. This cDNA was sequenced and computer searching against the GenBank revealed that the cDNA had more than 90% identity with the immunoglobulin kappa chain constant region (Ig κ-C).**

Discussion. **Our finding suggests that differentially expressed Ig κ-C gene only in AAA is a candidate gene that may play a pivotal role in the pathogenesis of AAA formation. The correlation of mRNA level and protein level is, however, not clear. Thus, to directly identify the role of Ig light chains in the pathogenic event of AAA, the further study comparing the level and kinds of expressed protein with the corresponding Ig κ-C gene will be required.**

Key Words: **aneurysm; aorta; differential expression genes; immunoglobulin kappa light chain; constant region.**

INTRODUCTION

A clinical feature of abdominal aortic aneurysm (AAA) is the progressive dilation of the aortic wall. The degradation of the extracellular matrix (ECM) components (collagen and elastins) does play an important role in the development of the aortic aneurysm [1, 2]. In previous reports, loss of elastin [3], increased collagen turnover [4], and elevated levels of matrix metalloproteinases (MMPs) [5, 6] have been detected in AAA compared with normal abdominal aortic wall (NA). MMPs are a family of related zinc metalloendopeptidase that function in the turnover of the ECM components [7, 8]. An important source of MMPs in AAA is the inflammatory cells [9], which are considered to participate in the immunopathogenesis of AAA. Infiltration of chronic inflammatory cells has been described in the wall of AAA [10]. Inflammation is probably the main process responsible for the development of changes in the extracellular matrix of the aortic wall leading to the destruction of elastin and the remodeling of collagen fibers.

The expression and function of the proteins participate in ECM degradation and chronic aortic wall inflammation of AAA, may be directly or indirectly related with the mRNA expression level. Thus, a number of the researches into the causes of AAA have focused on the alteration of gene expression and the results have been recently reported [11–14]. The difference in gene expression in the disease and normal groups must be good targets to identify the genetic factors associ-

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ated with the pathogenic aspect of a disease. Currently, differential display reverse transcription-polymerase chain reaction (DDRT-PCR) technique is a useful method for identifying the genes of diseases. However, this technique has a setback in that many artifact cDNA products present abnormal polymerase chain reaction (PCR) amplification. Therefore, it is difficult to select authentic cDNAs. To solve this setback, we used the annealing control primer (ACP) system, which was recently developed to identify only authentic genes. This technology improves the specificity and sensitivity of PCR amplification so that it enables us to find only authentic PCR products.

The present study was designed to identify differentially expressed genes in AAA compared to NA and aortic occlusive disease (AOD, Leriche's syndrome) using the ACP method.

METHODS

Preparation of Specimens

This study was approved by the Institutional Review Board of our institution and the specific patient consent was obtained for this study.

Three groups of human abdominal aorta were examined. The pieces of abdominal aortic wall were obtained from 10 AAA and three AOD patients during elective surgical repair. The inflammatory type of AAA was excluded from this study. The pieces of NA were obtained from five transplant brain death donors at the time of organ harvest. Tissue specimens were frozen in liquid nitrogen immediately on procurement and stored at −70°C in a nitrogen-tank.

Total RNAs Isolation and Differential Display of cDNAs

Aortic tissues were pulverized under liquid nitrogen, and total RNAs were isolated with Trizol reagent (Life Technologies, Inc., Grand Island, NY), as described before [15].

We performed differential display of cDNAs using the recently developed ACP system (GeneFishing DEG kit, Seegene Corp., Korea) [16, 17]. Total RNAs were used for the synthesis of first-strand cDNAs by reverse transcriptase. Reverse transcription was performed by mixing 3 μg total RNA, 10 μM anchor ACP-T and RNase-free water. After the mixture was heated at 80°C for 3 min, the tube was chilled on ice for 2 min. The mixture was then added in 5 × reverse transcription buffer, 2 mM dNTP, 20 units of RNase inhibitor, and 100 units of reverse transcriptase and incubated at 42°C for 90 min. The tube was heated at 94°C for 2 min and chilled on ice for 2 min. Second-strand cDNA synthesis and subsequent PCR amplification were conducted in a single tube. Second-strand cDNA synthesis was conducted in a reaction mixture containing 50 ng of reverse-transcription-reaction mixture, PCR buffer, 25 mM $MgCl_2$, 2 mM dNTP, 10 μM anchor ACP-T, 5 μM of 100 kinds of arbitary 37-mer nucleotide (A1~A100), 2.5 unit of Taq polymerase (Promega, Madison, WI), and adjusted 50 μl of final volume with distilled water. One round of first-stage PCR was done at 94°C for 3 min and at 50°C for 3 min, 72°C for 1 min and 72°C for 40 s and 40 rounds of second-stage PCR were done at 94°C for 40 s, 65°C for 40 s, and 72°C for 40 s and extension was done at 72°C for 5 min. PCR reaction was carried out using ABI GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA). The amplified cDNAs were then separated on 2% agarose gel stained with EtBr.

Sequencing of the Differentially Expressed cDNAs

Amplicons exhibiting differential expressions following the reverse Northern screen were sequenced using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kits (version 2.0) from Perkin-Elmer (Boston, MA) and automatic sequencer. The sequences were compared with the National Center of Biotechnology Information non-redundant sequence database using the Basic Local Alignment Search Tool (BLAST) program.

Recovery of cDNA Probes

Difference of cDNA expression pattern was screened and the band corresponding to the cDNA was excised from the agarose gel. Each gel slice was incubated in 100 μl of water at room temperature for 10 min and boiled for 15 min to release the PCR products. The cDNA was recovered by ethanol precipitation in the presence of 0.3 M sodium acetate and 5 μl of 10 mg/ml glycogen.

Northern Blot Analysis and Restriction Enzyme Digestion

Recovered cDNA probe was used for restriction enzyme digestion and Northern hybridization. The probe was incubated at 37°C for 60 min with 1 unit of *Sac I* enzyme. Northern hybridization was performed as described previously with minor modification [18]. Total RNA (15 ug) was denatured at 55°C for 15 min. The denatured RNA was applied to a 1.2% formaldehyde/agarose gel and electrophoresed in 1 × MOPS buffer (pH 7.0). The result was confirmed by a standard probe using β-actin (417 bp) targets.

RESULTS

In ACP system, a hundred arbitrary 37-mer primers having a 10 target nucleotide sequence (10-mer) at their 3′-end core positions were screened against one anchored oligo-dT primers. A differential display reaction yielded between 5 and 15 discrete cDNA bands ranging from 100 bp to 2 kb, which can be detected on agarose gels.

One differentially expressed cDNA band was detected in AAA but not in NA (Fig. 1A). This cDNA was sequenced and computer searching against the GenBank revealed that the cDNA had more than 90% identity with Ig kappa chain constant region (Ig κ-C). According to the web cutter software, it was known that *Sac I* enzyme site was located on the Ig κ-C sequences. The fact that one differentially expressed cDNA had a single band was identified by *Sac I* restriction enzyme digestion (Fig. 1B). The expression of Ig κ-C gene was confirmed by Northern blot analysis. Results from the Northern blotting shown that the Ig κ-C mRNA was presented in all of the AAA (Fig. 1C). We, also, examined the Ig κ-C gene expression in AOD using the same arbitrary ACP primer as used the amplification of Ig κ-C gene. The Ig κ-C gene was not expressed in AOD (Fig. 2). This indicated that the Ig κ-C gene expression was unique only in AAA.

DISCUSSION

Infiltration of chronic inflammatory cells has been described in the wall of AAA [10] along with Russell