Cloning and analyzing of the cDNA sequence of N-terminal region and C-terminal region of zinc finger protein (ZFP580) gene☆

Yebin Zhu[1,2], Wencheng Zhang[1,*]

[1]*Physiology and Pathology Laboratory*, *Medical College of Armed Police Force*, *Tianjin* 300162, *China*; [2]*HeBei Medical University*, *Shijiazhuang* 050017, *China*

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Abstract

Objective. To clone and analyze the cDNA sequence of N-terminal region and C-terminal region of zinc finger protein (ZFP580) gene from rat cerebellum. *Methods*. Total RNA was extracted from rat cerebellum. The two cDNA fragment encoding the N-terminal region and C-terminal region of *ZFP580* was amplified by RT-PCR with specific primers, cloned into pMD18-T-Easy vector and then sequenced, respectively. *Results*. The two fragments were obtained by RT-PCR successfully. DNA sequencing result showed that the two fragments were exactly consistent with the sequence reported in gene bank. *Conclusion*. The results showed that the N-terminal region and C-terminal region of *ZFP580* was successfully cloned, which lays a reliable foundation for further research of genetically engineered drugs and the gene therapies for atherosclerosis. [Life Science Journal. 2008; 5(1): 68 – 73] (ISSN: 1097 – 8135).

Keywords: ZFP580; gene cloning; atherosclerosis; C_2H_2 Zinc Finger

1 Introduction

Atherosclerosis (AS) is a major reason resulted in cardiovascular disease and stroke. Nowadays, more and more investigators consider that the etiology of AS involves multiple genetic and environmental factors. In addition, it was believed that the low-density lipoprotein (LDL), especially Oxidized LDL (Ox-LDL) is a significant and independent risk factor triggering AS. Improving AS resistance by genetic engineering was thought to be a new and efficient curing strategy. Zinc finger protein (ZFP580) gene originated from rat or mouse was proved to be a novel gene related to LDL[1,2], which would be a very potential gene for the exploring the gene curing of cardiovascular disease and stroke. Many reports have demonstrated that the N-terminal region and C-terminal region of ZFP take different roles for the regulation of down-stream gene transcription, and the procine (Pro) redundancy in N-terminal could be an activator or inhibitor for different resources ZFP. C_2H_2 structure in the C-terminal could bind with GC conservative regions in the cis-acting element of regulating genes. Therefore, the aim of this study is to obtain and analyze the sequence of N-terminal and C-terminal region of ZFP from rat cerebellum. Cloning the two fragments of Zinc Finger Protein (ZFP580) Gene successfully is significant to the next study of genetic engineering. The polypeptide of the N-terminal of ZFP580 would be used as antibody to explore a new curing drug for the AS, and the C-terminal of ZFP580 would be used as probe to precise targeting to a particular gene or genes of interest.

2 Materials and Methods

2.1 Plasmids, bacterial strains and culture conditions

Escherichia coli (*E. coli*) strain DH5a was used as recipient strain for plasmid transformation and amplification. The pMD18-T purchased from Takara (Dalian, China) was used for cloning of PCR products. The Luria-Bertani medium was used for culture of DH5a, with aeration at 37

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*Corresponding author. Tel: 86-22-6057-8250; Email: wenchengzhang@yahoo.com

°C in Luria broth (LB). Ampicillin was added to the medium (100 μg/ml) when DH5a harboring the recombinant plasmid was selected.

2.2 Reagents

All reagents were of analytic grade. Trizol extraction solution, gel retrieval kit and plasmid extraction kit were purchased from Ding Guo (Beijing, China) and were used according to the instructions of the manufacturer. Reverse transcriptase kit and T-A clone kit were obtained from Takara (Dalian, China). Endonucleases such as *Xho*I, *Bgl*II were obtained from TOYOBO (Ding Guo, China).

2.3 The Cloning of N -terminal region and C-terminal region of *ZFP580*

2.3.1 Preparation of total RNA. The fresh tissue of rat cerebellum frozen at liquid nitrogen was ground after adding Trizol solution. RNA was extracted according to the kit instructions. The total RNA isolated was treated with DNase I (RNase-free, Gibco BRL, USA) to remove residual DNA. The integrity of total RNA was examined by electrophoresis agarose gel containing formaldehyde.

2.3.2 RT-PCR reaction. Reverse transcription was performed starting from total RNA by using oligo(dT) primer. Total RNA (1 μg) was reversely transcribed with AMV reverse transcriptase by following the manufacturer's protocols. The N-terminal region and the C-terminal region of cDNA fragment of *ZFP580* was obtained via reverse transcription polymerase chain reaction (RT-PCR) with primers based on the sequence of rat ZFP580 cDNA published by GenBank. A pair of primers (p1 and p2) was designed to amplify the C-terminal (252 bp) fragment by using Primer Primer 5.0 and DNA club. Another couple of primers (p3 and p4) were designed to amplify the N-terminal region (264 bp). These primers were listed as follows: P1 (forward strand): 5′ TCG AGATCT CGCAA GGGCT ACAGC TGCC 3′. P2 (reverse strand): 5′ CTA CTCGAG TTAGT GCAGG CGCAC GTG 3′. P3 (forward strand): 5′ ACT AGATCT ATGCT GCTGC TGCCG CCG 3′. P4 (reverse strand): 5′ ATA CTCGAG AGGAC CTGGC TCTCC CGG 3′. *Xho*I and *Bgl*II endonuclease sites (underlined) were included in these four primers to facilitate subsequent cloning steps. PCR was performed according to the methods described in the Molecular Cloning Laboratory Manual[3]. PCR product is stored at 4 °C.

2.3.3 Construction and identification of plasmid pMD18-T-N-terminal and pMD18-T-C-terminal. The target fragment was purified and used in the ligation. The ligation reaction was carried out at 16 °C for 1 h according to the instructions of the T-A clone kit. The transformation was performed as described previously, Single white clone was selected and its plasmid DNA was prepared[3]. Plasmids were extracted according to the instructions of plasmid extraction kit, and digested respectively by restriction enzyme *Xho*I and *Bgl*II to select recombinants. Restriction enzymes digestion products are separated by electrophoresis on 1% agarose gel containing ethidium bromide and analyzed by a video imaging system. Positive recombinant clone is sent to Takara Company to be sequenced by using the dideoxy chain-termination method and confirmed by reading both strands.

2.4 Sequence analysis of the two fragments of *ZFP580* gene

Sequence analysis were done at the National Center for Biotechnology Information servers (www. ncbi. nlm. nih. gov/BLAST), and edited using the Primer 5, Clustalx and Danman programs.

3 Results

3.1 Obtaining the N-terminal region and C-terminal region of *ZFP580* gene

The RNA product from rat cerebellum tissue is separated by eletrophoresis (Figure 1). The ratio A260/A280 and A260/A230 of RNA sample was all above 2.0, which demonstrated RNA quantity and purity were well and used in the following RT-PCR. The fragments encoding of the N-terminal region and C-terminal region of *ZFP580* gene were amplified *via* polymerase chain reaction (PCR), according to the procedure described in the methods. The results were showed in Figure 2. The amplified partial cDNA were 264 bp and 252 bp, respectively. The result was in accordance with expected molecular weight.

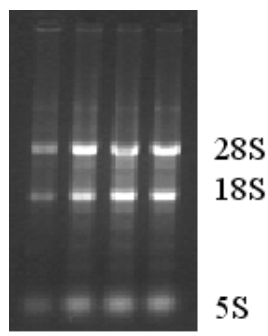

Figure 1. RNA agarose electrophoresis of rat cerebellum (containing formaldehyde).

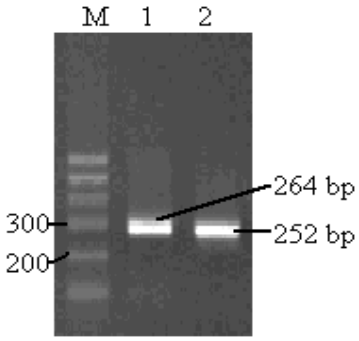

Figure 2. Agarose electrophoresis of RT-PCR products. M: 100 bp DNA ladder; Lanes 1 and 2: The N-terminal region and the C-terminal region of *ZFP580* gene.

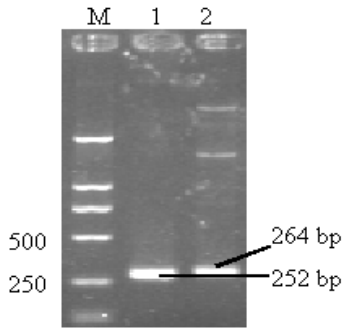

Figure 3. pMD18-T-N-terminal and pMD18-T-C-terminal was identified with PCR. M: DL 2000 DNA Marker; Lane 1: pMD18-T-C-terminal as template; Lane 2: pMD18-T-N-terminal as template.

3.2 Identification of recombinant of plasmid pMD18-T-N-terminal and pMD18-T-C-terminal

5 μl of the ligated product was used to perform transformation in *E. coli* DH5α competent cells, transformants were screened using LB agar with Ampicillin (100 μg/ml), the plasmids were extracted from the transformants, then PCR and enzyme digestion was carried out to screen positive clones. PCR was performed with the extracted plasmid as template, with P1 and P2, P3 and P4 as primer to amplify intent fragments, respectively. The result of agarose gel electrophoresis showed that the PCR products were 252 bp and 264 bp fragments, the size is in coincidence with anticipation (Figure 3). In addition, the results that the recombinant plasmids was double digested with *Xho*I and *Bgl*II, showed an insert of about 264 bp and 252 bp fragment, respectively, which were in consistence with the theoretical size of the N-terminal and C-terminal region of *ZFP580* (Figure 4). Above data indicated that the two sequences were constructed into the pMD18-T vector successfully. Positive recombinant clone is named as pMD18-T-N-terminal and pMD18-T-C-terminal, respectively.

3.3 Sequence analysis of the two fragments of *ZFP580* gene

The N-terminal region and the C-terminal region of *ZFP580* were compared homology with nucleotide sequence database of GenBank. The results are showed as Figures 5 and 6. The amino acids sequence was deduced, and N-terminal region and C-terminal region of *ZFP580* encode 88 and 84 amino acids, respectively (Figures 7 and 8).

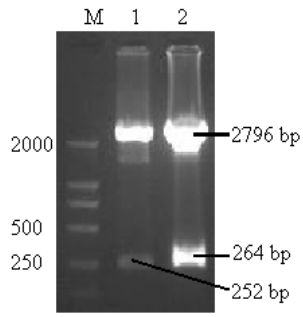

Figure 4. pMD18-T-N-terminal and pMD18-T-C-terminal was identified with *Xho*I and *Bgl*II digestion. M: DL 2000 DNA Marker; Lane 1: pMD18-T-C-terminal; Lane 2: pMD18-T-N-terminal.

4 Discussion

In this study, we, for the first time, successfully cloned the N-terminal region and C-terminal region of ZFP580 by PCR from rat cerebellum tissue. And the pMD18-T-N-terminal and pMD18-T-C-terminal recombinants were constructed with success.

The multiple-sequence alignment of N-Terminal demonstrated that the ZFP580 N-terminal cloned is completely consistence with Rattus norvegicus ZFP580 (predicted) sequence, and shows high conservation with ZFP580 from different sources. The identities were 99%, 92% and 91% with zinc finger protein 580 gene reported from *Mus musculus*, *Homo sapiens* and *Canis* familiaris similar to zinc finger protein 580, respectively (Figure 5). Compared with ZFP580 from *Mus musculus* and *Homo sapiens*, the identities of C-terminal nuclear sequence were 98% and 90%, respectively (Figure 6).

Figure 5. A multiple-sequence alignment of *ZFP580* N-terminal from human and mouse. Abbreviations are explained as follows: *ZFP580* from rat cerebellum, *Rattus norvegicus*, *Mus musculus*, *Homo sapiens* (*ZNF580*) and *Canis familiaris* similar to zinc finger protein, respectively.

The result of nuclear sequence blast of the two fragments of ZFP580 showed that the two fragments are conservative. There are no obvious evolutions of ZFP580 gene between rat and human. A strong selective pressure in the course of evolution maintains conservation and this suggests that the two fragments of the ZFP580 gene must taken very important and similar responsibilities for the metabolism regulation.

The result of amino acids sequence analysis of zinc finger protein online demonstrated that the ZFP580 has two domains. The comparison of amino acid sequence indicates that the N-terminal and the C-terminal of ZFP580 cloned encode N- and C-terminal domain, respectively, which is similar to the size of amino acids of zinc finger protein reported in the database. And the N-terminal region between amino acids 5 – 88 is also remarkable rich in Pro residues, amounting to 28.6%, which is very near to the 28.8% reported in ZNF580 (Figure 7). The redundancy of Pro might play the role of activating transcription of targeting gene[1]. The C-terminal region between amino acids 94 – 172 include three high conserved C_2H_2 zinc finger domains, which could bind with the GC rich region in the promoter of down stream targeting gene[4], and include several protein kinase phosphorylation site, such as SHR, SHSD, THR, KRSS (Figure 8). More and more studies have demonstrated that protein phosphorylation is ubiquitous in many transcription factor of C_2H_2 type, and closely linking to the regulation of metabolism and immune response reaction. Yano[5] and our former[6] work have demonstrated that the ZNF580 is located at the nucleus. And typical characteristics of ZFP580 suggested that it was a significant transcription factor involving in regulating transcription of many different genes.

We wanted use Rat as a model to study the relationship between the ZFP580 and the initiation and development

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ZFP580    --------CGCAAGGGCTACAGCTGCCCAGAGTGCGCTCGTGTCTTTGCCAGCCCTCTGC 52
Mus       CAGGTCCTCGCAAGGGCTATAGCTGCCCAGAGTGCGCTCGTGTCTTTGCGAGCCCTCTGC 60
Homo      ----CCCTCGCAAGGGCTACAGCTGCCCGGAGTGCGCCCGTGTCTTTGCCAGCCCTCTGC 56
                ********** ******** ******** ********** **********

ZFP580    GGCTGCAGAGCCACCGTGTGTCGCACTCGGACCTCAAGCCCTTTACTTGTGGTGCTTGTG 112
Mus       GGCTGCAGAGCCACCGTGTGTCGCACTCGGACCTCAAGCCCTTTACTTGTGGTGCTTGTG 120
Homo      GGCTGCAGAGCCACCGCGTGTCGCACTCGGACCTCAAGCCCTTCACGTGCGGCGCCTGCG 116
          *************** ************************ ** ** ** ** ** *

ZFP580    GCAAAGCTTTCAAGCGCTCCAGCCACCTGTCGCGGCACCGCGCCACCCACCGTGCGCGCG 172
Mus       GCAAAGCTTTCAAGCGATCCAGCCACCTGTCGCGGCACCGCGCCACCCACCGTGCGCGCG 180
Homo      GCAAGGCCTTCAAGCGCTCCAGCCACCTGTCGCGGCATCGCGCCACGCACCGCGCCCGCG 176
          **** ** ******** ******************** ******** ***** ** ****

ZFP580    CTGGTCCACCTCACACCTGCCCACTCTGTCCACGCCGCTTCCAGGACGCCGCGGAGCTGG 232
Mus       CTGGTCCACCTCACACCTGCCCGCTCTGTCCACGCCGCTTCCAGGACGCCGCGGAGCTGG 240
Homo      CCGGGCCGCCGCACACCTGCCCGCTCTGCCCACGCCGCTTCCAGGACGCCGCGGAGCTGG 236
          * ** ** ** *********** ***** ********************************

ZFP580    CGCAACACGTGCGCCTGCACTAA----------- 255
Mus       CGCAGCACGTGCGCCTGCACTGAGCTCAGC---- 270
Homo      CGCAGCACGTGCGCCTCCACTAAGCTCGAGACCC 270
          **** *********** **** *
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Figure 6. A multiple-sequence alignment of ZFP580 C-terminal from mouse and human. Abbreviations are explained as follows: *ZFP580* from rat cerebellum, *Mus musculus* and *Homo sapiens*, respectively.

MLLLPPRPPHPRSSSPEVMDPPPPKTPPFPKAEGPSSTSSSVAGP
RPPRLGRHLLIDANGVPYTYTVQLEEEPRGPPQREATPGEPGP

Figure 7. Amino acid sequence of *ZFP580* N-terminal.

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          RKGYS
Finger1   CPECARVFASPLRLQSHRVSHSDLKPFT
Finger2   CGACGKAFKRSSHLSRHRATHRARAGPPHT
Finger3   CPLCPRRFQDAAELAQHVRLH
Consensus C   C   F     L   H   H
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Figure 8. The structure of Zinc finger of *ZFP580* C-terminal.

of AS, because the structure homology between ZFP580 and ZNF580. So the construction of plasmid pMD18-T-N-terminal lays a foundation for further preparing for multiple cloning antibody to exploring a new curing pathway for AS. Construction of plasmid pMD18-T-C-terminal provide the basis for further screening oligonuclease probes, which would detect more gene down-regulated by the ZFP580, and maybe find new targeting gene to cure AS.

The works including ours will give light to better understand the link between the structure and biological activity of the ZFP580 gene, and more following work is done, which will lay a solid foundation for diagnosing, safeguarding and curing for atherosclerosis.

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