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Study of Resistin gene expression in peripheral blood mononuclear cell and its gene polymorphism in a small range population*

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Abstract: Objective: To observe the expression of Resistin mRNA in peripheral blood mononuclear cells and its gene polymorphism in coding region in a small range population in Zhejiang Province of China. Methods: Eighty-three cases of type 2 diabetes mellitus and 53 healthy people were included. The expression of Resistin mRNA in peripheral blood mononuclear cells was detected by RT-PCR and semi-quantitative PCR assay. The sequencing work was done in Resistin cDNA and gene polymorphism was analyzed. Results: At the same condition, in 83 diabetes patients, Resistin mRNA was detected in 23 cases (11 males and 12 females). There was no Resistin mRNA expression in 53 healthy people. The ratio of PCR products between Resistin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was from 0.564 to 1.238, averaging 0.804±0.436. The sequence of Resistin cDNA is almost identical with each other and with that in GenBank with no single nucleotide polymorphism being found. Conclusion: Resistin mRNA is expressed in human peripheral blood mononuclear cells in some type 2 diabetes mellitus, but its expression is at a low level. Among the experiment population we did not find polymorphism phenomenon in Resistin coding region. The different individual's Resistin coding region is highly coincident.

Key words: Resistin mRNA, Peripheral blood mononuclear cells, Coding region, Gene polymorphism
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INTRODUCTION

Recently discovered as an endocrine organ, adipose tissue can secrete many cytokines such as Resistin, adiponectin and free fatty acids, and receives much attention in the study of insulin resistance and type 2 diabetes mellitus (T2DM). As a peptide hormone secreted by adipocyte, Resistin is considered to be the linkage between obesity and insulin resistance (Steppan *et al*., 2001). Most studies on Resistin investigate adipose tissue. In this experiment we detected the expression of Resistin mRNA in human peripheral blood mononuclear cells (PBMC) in a small range population in Zhejiang Province of China and analysed the polymorphism of the Resistin gene.

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MATERIALS AND METHODS

Sample selection

Serum samples were taken with permission from 83 cases of T2DM patients [48 males and 35 females, mean age (54.1±16.3) years] who fulfilled the World Health Organization criteria for T2DM, and 53 healthy people [33 males and 20 females, mean age (58.6±9.7) years] from June, 2005 to Dec., 2005.

Detection of Resistin mRNA in human PBMC with semi-quantitative RT-PCR analysis

Total RNA was extracted with Trizol reagent (BD Bioscience) from human PBMC isolated from 3 ml peripheral blood using lymphocyte separating reagent (Sigma Co., USA), and RNA was treated with RNase-free Dnase. First-strand cDNA was generated from 10 μl of total RNA in a 20 μl volume containing

10×dNTP, 50 mmol/L oligo (dT) primer, M-MLV (200 μl^{-1}) etc. Oligo (dT) primer was 5′-TTTTTTTT TTTTTTTTTT-3′, and the reaction duration was for 1 h in a 42 °C water bath. First-strand cDNA from reverse transcription was used for conventional PCR.

Conventional PCR was carried out in mixtures containing 10 μl of template DNA, 10×dNTP, 50 mmol/L primers, 5 U/μl Taq DNA polymerase, 2 mmol/L $MgCl_2$ etc. Denaturation was at 94 °C for 1 min, annealing at 55 °C for 45 s, polymerization at 72 °C for 30 s with 30 cycles, and ending with a single 5-min extension step at 72 °C. Primers were 5′-TCTAGCAAGACCCTGTGC-3′ and 5′-CAGGTT TATTTCCAGCTCC-3′.

To quantify mRNAs, we employed the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal standard and determinated the relative levels of specific mRNAs by comparing the ratio of PCR products. The PCR primers for GAPDH were 5′-TGGGGAAGGTGAA GGTCGGA-3′ and 5′-GGGATCTCGCTGCTCGAA GA-3′. Quantitative analyses of mRNA levels were performed using GelScan V. 5.02 (BioSciTec GmbH, Giessen, Germany) image analysing system.

Sequencing work

Resistin cDNA sequence was assayed by the Sanger dideoxy-mediated chain termination method using Automated DNA Sequencers ABI3700.

Statistical analysis

All data were expressed in as mean±*SD* with software SPSS11.0 being used to analyse the data. *t* test and Pearson correlation analysis were adopted.

RESULTS

There were 23 T2DM patients (11 males and 12 females) who showed expression of Resistin mRNA among the 83 cases (Fig.1). No Resistin mRNA was detected in the 53 healthy people. Semi-quantitative PCR showed that the ratio between Resistin gene and GAPDH (Fig.2) was from 0.564 to 1.238 and that the average was 0.804±0.436. Sequencing work was done in 23 positive cases. No single nucleotide polymorphism (SNP) was found after blastn. The detected Resistin gene order was almost identical with that in GenBank, with coincidence rate being as high as 98%.

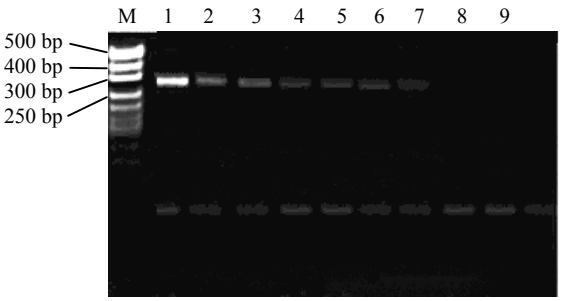

Fig.1 Expression of some samples of Resistin gene using RT-PCR
M: Maker; 1~7: Resistin gene; 8: Negative control; 9: Blank control

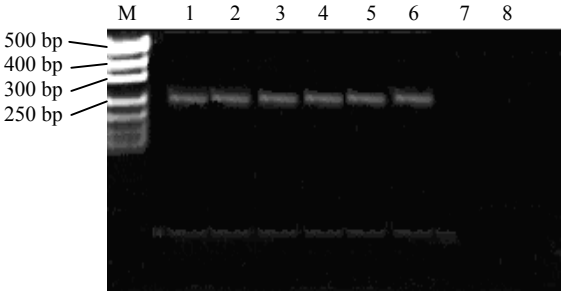

Fig.2 Expression of GAPDH using PCR method
M: Maker; 1~6: GAPDH; 7: Negative control; 8: Blank control

DISCUSSION

Human Resistin gene is located in No. 19 chromosome. Resistin mRNA has 476 basic radicals with their coding region having 326 basic radicals which code 108 amino acids (Steppan *et al*., 2001). Human Resistin gene was mainly expressed in various kinds of adipose tissue (Nogueiras *et al*., 2003; Minn *et al*., 2003), with its expression being regulated by many kinds of factors. There are reports that androgen (Ling *et al*., 2001) and growth hormone (Delhanty *et al*., 2002) can stimulate the expression of Resistin mRNA, while free fatty acid (Juan *et al*., 2001), rosiglitazone (Li and Lazar, 2002) and tumour necrosis factor (Fasshauer *et al*., 2001; Yang *et al*., 2005) can inhibit its expression. In 3T3-L1 adipocytes, Chung *et al*.(2006) found that PPARgamma activation represses the expression of the Resistin gene by modulating Sp1 activity, Sp1 binding site of the Resistin promoter (−122/−114 bp) was necessary and the

level of O-glycosylation of Sp1 was decreased in this repression.

We detected 136 people's expression of Resistin mRNA from PBMC in Zhejiang Province in China. Among the 136 people, there were 83 T2DM patients and 53 healthy people, 81 males and 55 females. Only 23 T2DM patients had expression of Resistin mRNA. The result of semi-quantitative PCR showed that the level of expression differed greatly between patients. Further, the expression of Resistin mRNA was at a low level among experimental people and most people had no expression. Those who had Resistin mRNA expression all were T2DM patients, and this might demonstrate that Resistin has a close relation with T2DM. Moreover, we also found that females had a higher ratio of Resistin mRNA expression than males (22%/13%). As a type of cytokine secreted by adipose cells, Resistin is distributed mainly in various kinds of adipose tissue. But it also can be found in pancreatic islets (Minn *et al*., 2003), trophoblastic cells in placental tissue (Yura *et al*., 2003), human macrophages (Patel *et al*., 2003) and leukemia cells (Yang *et al*., 2003). In the most recent study, Wiesner *et al*.(2006) put forward that brain injury, or an inflammatory stimulus, regulates the central expression of Resistin gene normally considered to be adipose tissue-specific. Kaser *et al*.(2003) found that human PBMC had expression of Resistin mRNA, and he even suggested human PBMC may be a major source of Resistin. Although we detected Resistin mRNA expression in human PBMC, the expression in our experiment is low. Some factors, such as the method adopted, difference of region and race, drugs that perhaps can influence Resistin, may contribute to this finding. Studies of Resistin expression in other regions and races are needed to learn more about Resistin.

Regarding Resistin gene polymorphism, our study found that in Japanese people, some SNPs such as −638G>A, −420C>G, −358G>A, −167C>T, +157C>T and +299G>A were detected in flanking region (Osawa *et al*., 2004), promoter region (Azuma *et al*., 2004) and introns (Osawa *et al*., 2002) of the Resistin gene. This SNP can increase RETN promoter activity and influence Resistin gene transcriptional activity, and thereby influence the expression of Resistin gene and protein (Osawa *et al*., 2004; Azuma *et al*., 2004). Korea scholar Cho *et al*.(2004) screened −537A>C and −420C>G in the promoter region of Resistin gene, and found that polymorphisms are major determinants of plasma Resistin concentrations in human. In Taiwan Province of China, Tan *et al*.(2003) studied 1102 Chinese type 2 diabetes patients and 743 subjects without diabetes. The Resistin 3'-untranslated region (UTR) +62G→A polymorphism was determined by PCR. They found that type 2 diabetes subjects had a lower frequency of Resistin gene 3'-UTR+62A allele (GG:GA/AA, 83.5%:16.5%) than the controls (GG:GA/AA, 75.1%:24.9%). These findings suggest that Resistin may play a role in the pathogenesis of type 2 diabetes and insulin resistance-related hypertension. In non-Asian race studies, Gouni-Berthold *et al*.(2005) repeated Tan's experiment. He found that in a German Caucasian population the +62G→A polymorphism of the Resistin gene is associated with hypertension but not with T2DM. Our experiment selected some people from Zhejiang Province of China and sequenced the coding region of Resistin gene. We found that the sequence of coding region was highly conservative although polymorphism might exist in other region such as promoter region, introns and 3'-UTR. This point is similar with that of Osawa *et al*.(2002). In his experiment, sequences for 24 Japanese type 2 diabetic patients were initially analyzed using PCR direct sequencing. Three SNPs (−167C>T, +157C>T and +299G>A) were found in the introns, but none were present in the coding regions. In another study, Sentinelli *et al*.(2002) analyzed the coding sequence of the three exons of the Resistin gene, together with its 5' regulatory region and 3'-UTR, by single-strand conformation polymorphism (SSCP) in 58 type 2 diabetic subjects, 59 obese subjects, and 60 normal subjects. Only one sequence variant (G1326C) was detected in the 3'-UTR of exon 3.

As a promising candidate gene for type 2 diabetes, Resistin is not fully recognized by us. A lot of work remains for us to understand its physiological functions with more large scale experiments being needed to confirm the conclusion we put forward.

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