Difference in gene expression of macrophage between normal spleen and portal hypertensive spleen identified by cDNA microarray

Feng Yan, Xiao-Min Wang

Feng Yan, Postdoctoral Station, Department of Hepato-Biliary Surgery, Zhongshan Hospital, Xiamen University, Xiamen 361004, Fujian Province, China
Xiao-Min Wang, Department of Hepato-Biliary Surgery, Zhongshan Hospital, Xiamen University, Xiamen 361004, Fujian Province, China
Supported by the National Natural Science Foundation of China, No. 30170909
Correspondence to: Dr. Feng Yan, Postdoctoral Station, Department of Hepato-Biliary Surgery, Zhongshan Hospital, Xiamen University, Xiamen 361004, Fujian Province, China. yanyisheng_ricky@tom.com
Telephone: +86-592-2292517 Fax: +86-592-2212328
Received: 2007-03-13 Accepted: 2007-04-07

Abstract

AIM: To identify the difference in gene expression of microphage (Mϕ) between normal spleen and portal hypertensive spleen using cDNA microarrays and find new gene functions associated with hypersplenism in portal hypertension.

METHODS: The Biostar-H140s chip containing 14112 spots of cDNAs were used to investigate the difference of the expression. The total RNA extracted from macrophages isolated from both normal spleen and portal hypertensive spleen was reversely transcribed to cDNA with the incorporation of fluorescent (cy3 and cy5) labeled dCTP to prepare the hybridization probes. After hybridization, the gene chip was scanned for the fluorescent intensity. The differentially expressed genes were screened. That was repeated three times, and only the genes which had differential expression in all three chips were considered to be associated with hypersplenism in portal hypertension.

RESULTS: Eight hundred and ninety-six, 1330 and 898 genes were identified to be differentially expressed in three chips, respectively. One hundred and twenty-one genes (0.86%) were identified to be differentially expressed in all three chips, including 21 up-regulated genes and 73 down-regulated genes. The differentially expressed genes were related to ionic channel and transport protein, cyclin, cytoskeleton, cell receptor, cell signal conduct, metabolism, immune, and so on. These genes might be related to the hypersplenism in portal hypertension.

CONCLUSION: The investigations based on cDNA microarray can screen differentially expressed genes of macrophages between normal spleen and portal hypertensive spleen, thus may provide a new idea in studying the pathogenesis of hypersplenism in portal hypertension.

© 2007 The WJG Press. All rights reserved.

Key words: Hypersplenism; Macrophage; cDNA microarray

INTRODUCTION

It is reported that, compared with the macrophage (Mϕ) in normal spleen, the Mϕ in portal hypertensive spleen has a large amount of acid phosphatase, lysosome and pseudopodium, and can destruct much more erythrocytes and thrombocytes. This proved that the destruction of hemocytes by Mϕ of spleen plays an important role in the development of hypersplenism in portal hypertension [1,2].

Our previous studies suggested that phagocytosis of Mϕ was augmented in hypersplenism in portal hypertension; however, the specific mechanisms are not clear. In this study, cDNA microarrays were used to detect the difference in gene expression of Mϕ between normal spleen and portal hypertensive spleen and find new gene functions associated with hypersplenism in portal hypertension in an attempt to explore the pathogenesis of hypersplenism in portal hypertension.

MATERIALS AND METHODS

Materials

The excised human spleen specimens used in this study were provided with the approval of the hospital authorities. The experimental group included 3 cases of excised human spleen of portal hypertension and hypersplenism (all 3 cases had chronic hepatitis B), and the
control group included 2 cases of excised human spleen of traumatic splenic rupture.

**Mφ isolation and purification and total RNA extraction**
Mφ was isolated and purified by adherent culture[3]. Total RNA was extracted from Mφ by the TRIzol method[4].

**Construction of cDNA microarray**
The Biostar-H140s cDNA microarray provided by Shanghai BioStar Genechip Inc., consists of a total of 14112 human genes. The cDNA inserts were amplified using the polymerase chain reaction (PCR) with universal primers, and then purified according to standard method. All PCR products were examined by agarose gel electrophoresis to ensure the quality. Then the amplified PCR products were dissolved in a buffer solution. The solution with amplified PCR products were spotted onto silylated slides (TeleChem International, USA) using a Cartesian PixSys 7500 motion control robot (Cartesian Technologies, USA). Glass slides with spotted cDNA were hydrated for 2 h in 700 mL/L humidity, dried for 0.5 h at room temperature, and UV crosslinked (65 mj/cm). They were further processed at room temperature by soaking in 2 g/L sodium dodecyl sulfate (SDS) for 10 min, in distilled H2O for 10 min, and 2 g/L sodium borohydride (NaBH4) for 10 min. The slides were dried again and ready for use.

**Probe preparation**
The fluorescent cDNA probes were prepared through reverse transcription and then purified according to the protocol of Schena[5]. The total RNA of Mφ was extracted from 2 cases of normal spleen respectively, and then was mixed as the control group. The total RNA of Mφ was extracted from 3 cases of portal hypertensive spleen respectively, and each case was treated as the experimental group. The probes from the total RNA of control group was labeled with Cy3-dUTP, while those from the total RNA of experimental group were labeled with Cy5-dUTP. The probes were then mixed, precipitated and resolved in a hybridization buffer.

**Hybridization and washing**
Microarrays were pre-hybridized with hybridization solution containing 0.5 g/L denatured salmon sperm DNA at 42°C for 6 h. Fluorescent probe mixtures were denatured at 95°C for 5 min, and the denatured probe mixtures were applied onto the pre-hybridized chip under a cover glass. Chips were hybridized at 42°C for 16-18 h. The hybridized chips were then washed at 60°C for 10 min each in the mixture of 5 mL/L solution 1 and 20 mL/L solution 2, and 50 mL/L solution 3, then dried at room temperature for scanning (all reagents used in this procedure were contained in the Chip Hybridization Kit provided by Shanghai BioStar Genechip Inc.).

**Detection and analysis**
The chips were scanned with a ScanArray 4000 (Packard Biochip Technologies, USA) at two wavelengths to detect emission from both Cy3 and Cy5. The acquired images were analyzed using QuantArray software (Packard Biochip Technologies, USA). Ratios of Cy5 to Cy3 were computed for each location on each microarray. Overall intensities were normalized with a correction coefficient obtained using the ratios of 96 housekeeping genes in each chip. The intensities of each spot at the two wavelengths represent the quantity of Cy3-dUTP and Cy5-dUTP, respectively, hybridized to each spot. Thus, the ratio of each spot represents the ratio of mRNA expression abundance between the gene of Mφ in normal spleen and portal hypertensive spleen. The detection results were described in both scanned microarray images and microarray scatter plots. That was repeated three times, and only the genes that had differential expression in all three chips were considered associated with hypersplenism in portal hypertension.

**RESULTS**

**Scanned microarray images**
In the scanned microarray images (Figure 1), red points represent the higher expression genes of Mφ in portal hypertensive spleen than those in normal spleen, green points represent the lower expression genes, and yellow points represent the genes that have no change in expression. The hybridization signal of chips is distinct and balanced, indicating that the results are reliable. Compared with the genes of Mφ in normal spleen, a few genes of Mφ in portal hypertensive spleen were highly expressed, some were lowly expressed, however most genes showed no change in expression.

**Microarray scatter plots**
As indicated in the microarray scatter plots (Figure 2), most genes show a concentrated pattern surrounding the diagonal (red points), which means that the ratios range from 0.5 to 2.0, and there is no difference in the gene expression between normal spleen and portal hypertensive spleen. However, the other genes are away from the diagonal (yellow points), indicating that the ratios are beyond the range of 0.5-2.0, and the difference in the expression of those genes is not significant between normal spleen and portal hypertensive spleen.
There were 896, 1330 and 898 genes identified to be differentially expressed in three chips, respectively; 121 genes (0.86%) were differentially expressed in all three chips, including 95 genes which could be found in the GenBank, the other 26 genes were not reported and probably were unidentified novel genes. Among 95 known genes, 1 gene (GenBank No: NM_012218) was related to hepatitis B, and the other 94 genes might be those that were differentially expressed between the Mϕ in normal spleen and the Mϕ in portal hypertensive spleen, including 21 up-regulated known genes and 73 down-regulated known genes. Ten differentially expressed genes that were up-regulated in macrophages of portal hypertensive spleen and find new gene functions associated with hypersplenism in portal hypertension in an attempt to explore the pathogenesis of hypersplenism in portal hypertension. No similar study has been reported until now.

In order to obtain enough amounts of total RNA and eliminate individual variation, the total RNA of Mϕ from 2 cases of normal spleen respectively was mixed as the control group, and then was matched with that of 3 cases of the experimental group to 3 match-pairs for the cDNA microarray analysis. The differentially expressed genes were found to be related to ion channel and transport protein, cyclin, cytoskeleton, cell receptor, cell signal conduct, metabolism, immune, and so on.

**DISCUSSION**

Since the microarray analysis was first reported by Schena\[1\] in 1995, gene chips have been widely used in studying the functions of genes. The results of this study proved that gene chips can successfully profile changes in gene expression on a genomic scale with low consuming, high sensitivity and high-flux. In this study, cDNA microarrays were used to detect the difference in gene expression of Mϕ between normal spleen and portal hypertensive spleen and find new gene functions associated with hypersplenism in portal hypertension in an attempt to explore the pathogenesis of hypersplenism in portal hypertension. No similar study has been reported until now.
PRKCD encoding protein kinase C delta\(^7\) was found down-regulated in the M\(\Phi\) of portal hypertensive spleen. Protein kinase C delta plays an important role in regulating IL-13-induced 15-lipoxygenase (15-LO) expression in human monocytes and subsequently modulates the inflammatory responses mediated by 15-LO products\(^8\). Besides, protein kinase C delta is related to monocytic differentiation\(^9,10\). These findings indicate that PRKCD has a close relationship with the function of human monocytes (including M\(\Phi\)). The mature dendritic cell (DC) is considered to be the most potent antigen-presenting cell. Regulation of the DC, particularly its survival, is therefore critical. Bertho \textit{et al}\(^1\) found that MHC class II-mediated apoptosis of mature DC is produced by activation of the protein kinase C delta isoenzyme. Thrombin can stimulate the production of vascular adhesion molecule-1 (VCAM-1) in endothelial cells, however, it is found to be mediated by the signaling pathways involved with protein kinase C delta\(^12\). These findings indicate that PRKCD plays an important role in inducing apoptosis and producing cytokines. However, the effects of down-regulated PRKCD on the M\(\Phi\) of portal hypertensive spleen remain to be further investigated. IL-1 is an important mediator of inflammation and tissue damage in multiple organs in both experimental animal models and humans\(^13-15\). The balance between IL-1 and IL-1Ra (interleukin 1 receptor antagonist, IL-1Ra) in local tissues plays an important role in the susceptibility to and severity of many diseases\(^16,17\). Treatment of rheumatoid arthritis (RA) with daily subcutaneous injections of recombinant IL-1Ra protein has been shown to be efficacious. Gene therapy with IL-1Ra is being evaluated for the treatment of RA and other human diseases\(^18\). IL1RN encoding IL-1Ra was found down-regulated significantly (the average ratio was 0.179) in the M\(\Phi\) of portal hypertensive spleen. This leads to the imbalance between IL-1 and IL-1Ra, and it might be related to the pathogenesis of hypersplenism in portal hypertension, but the specific mechanisms need to be further studied.

ASK encoding activator of S phase kinase was found up-regulated in the M\(\Phi\) of portal hypertensive spleen. Cdc7-Dbf4 kinase complexes, conserved widely in eukaryotes, play essential roles in initiation and progression of the S phase. Cdc7 kinase activity fluctuates during cell cycle, and this is mainly the result of oscillation of expression of the Dbf4 subunit. Yamada \textit{et al}\(^19\) had isolated and characterized the promoter region of the human ASK gene encoding Dbf4-related regulatory subunit for human Cdc7 kinase, and identified one ASK promoter segment, which was sufficient for mediating growth stimulation. In the M\(\Phi\) of portal hypertensive spleen, the up-regulation of ASK may lead to the activity enhancement (including phagocytosis) of M\(\Phi\), resulting in the pathogenesis of hypersplenism in portal hypertension. Phosphatidylinositol 3-kinase (PIK3) is a key step in the metabolic actions of insulin. One 85 KDa regulatory subunit of PIK3 is encoded by PIK3R1 (phosphoinositide-3-kinase, regulatory subunit 1). It was proved that the expression of PIK3R1 was associated with alterations in glucose/insulin homeostasis\(^20\). In our study, PIK3R1 was found up-regulated significantly in the M\(\Phi\) of portal hypertensive spleen, indicating that more insulin existed and the glycometabolism was enhanced in M\(\Phi\). Furthermore, enhancement of glycometabolism is regarded as an index of enhanced cell functions, therefore we presume that the up-regulation of PIK3R1 may cause the functional enhancement of M\(\Phi\) in spleen. However, the possible molecular mechanisms remain undiscovered.

Many differentially expressed genes of M\(\Phi\) between normal spleen and portal hypertensive spleen have been successfully screened by cDNA microarrays, providing clues and target genes in studying the molecular mechanisms of pathogenesis of hypersplenism in portal hypertension. However, the implication of the gene expression needs to be further investigated.

**REFERENCES**

1. Yongxiang W, Zongfang L, Guowei L, Zongzheng J, Xi C, Tao W. Effects of splenomegaly and splenic macrophage activity in hypersplenism due to cirrhosis. \textit{Am J Med} 2002; \textbf{113}: 428-431
2. Li ZF, Zhang Y, Gao J, Zhang PJ, Wang JX, Liu XG. Expression and significance of Toll-like receptor 4 of splenic macrophage in patients with hypersplenism due to portal hypertension. \textit{Zhonghua Yi Xue Za Zhi} 2004; \textbf{84}: 1085-1091
3. Yan F, Li ZF, Zhang S, Yang JH, Li AM, Liu XG. Isolation and purification of macrophages from human spleen. \textit{Xi’an Jiaotong Daxue Xuebao} 2004; \textbf{25}: 452-455
4. Yan F, Li ZF, Su QH, Ma SHY, Cao G, Zhang S. Extraction and productivity of total RNA in macrophages isolated from human spleen. \textit{Zhonghua Shiyuan Waike Za Zhi} 2004; \textbf{22}: 176-177
5. Zhong WD, He HC, Bi XC, Ou RB, Jiang SA, Liu LS. cDNA macroarray for analysis of gene expression profiles in prostate cancer. \textit{Chin Med J (Engl)} 2006; \textbf{119}: 570-573
6 Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995; 270: 467-470

7 Huppi K, Siwarski D, Goodnight J, Mischak H. Assignment of the protein kinase C delta polypeptide gene (PRKCD) to human chromosome 3 and mouse chromosome 14. *Genomics* 1994; 19: 161-162

8 Xu B, Bhattacharjee A, Roy B, Feldman GM, Cathcart MK. Role of protein kinase C isoforms in the regulation of interleukin-13-induced 15-lipoxygenase gene expression in human monocytes. *J Biol Chem* 2004; 279: 15954-15960

9 Liu H, Keefer JR, Wang QF, Friedman AD. Reciprocal effects of C/EBPalpha and PKCdelta on JunB expression and monocytic differentiation depend upon the C/EBPalpha basic region. *Blood* 2003; 101: 3885-3892

10 Suh KS, Tatunchak TT, Crutchley JM, Edwards LE, Marin KG, Yuspa SH. Genomic structure and promoter analysis of PKCdelta. *Genomics* 2003; 82: 57-67

11 Bertho N, Blancheteau VM, Setterblad N, Laupeze B, Lord JM, Dréno B, Amiot L, Charron DJ, Fauchet R, Mooney N. MHC class II-mediated apoptosis of mature dendritic cells proceeds by activation of the protein kinase C-delta isoenzyme. *Int Immunol* 2002; 14: 935-942

12 Minami T, Abid MR, Zhang J, King G, Kodama T, Aird WC. Thrombin stimulation of vascular adhesion molecule-1 in endothelial cells is mediated by protein kinase C (PKC)-delta-NF-kappa B and PKC-zeta-GATA signaling pathways. *J Biol Chem* 2003; 278: 6976-6984

13 Firestein GS, Berger AE, Tracey DE, Chosay JG, Chapman DL, Paine MM, Yu C, Zvaifler NJ. IL-1 receptor antagonist protein production and gene expression in rheumatoid arthritis and osteoarthritis synovium. *J Immunol* 1992; 149: 1054-1062

14 Koch AE, Kunkel SL, Chensue SW, Haines GK, Strieter RM. Expression of interleukin-1 and interleukin-1 receptor antagonist by human rheumatoid synovial tissue macrophages. *Clin Immunol Immunopathol* 1992; 65: 23-29

15 Malyak M, Swaney RE, Arend WP. Levels of synovial fluid interleukin-1 receptor antagonist in rheumatoid arthritis and other arthropathies. Potential contribution from synovial fluid neutrophils. *Arthritis Rheum* 1993; 36: 781-789

16 Beaulieu AD, McColl SR. Differential expression of two major cytokines produced by neutrophils, interleukin-8 and the interleukin-1 receptor antagonist, in neutrophils isolated from the synovial fluid and peripheral blood of patients with rheumatoid arthritis. *Arthritis Rheum* 1994; 37: 855-859

17 Roux-Lombard P, Modoux C, Vischer T, Grassi J, Dayer JM. Inhibitors of interleukin 1 activity in synovial fluids and in cultured synovial fluid mononuclear cells. *J Rheumatol* 1992; 19: 517-523

18 Arend WP. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev* 2002; 13: 323-340

19 Yamada M, Sato N, Taniyama C, Ohtani K, Arai K, Masai H. A 63-base pair DNA segment containing an Sp1 site but not a canonical E2F site can confer growth-dependent and E2F-mediated transcriptional stimulation of the human ASK gene encoding the regulatory subunit for human Cdc7-related kinase. *J Biol Chem* 2002; 277: 27668-27681

20 Almind K, Delahaye L, Hansen T, Van Obberghen E, Pedersen O, Kahn CR. Characterization of the Met326Ile variant of phosphatidylinositol 3-kinase p85alpha. *Proc Natl Acad Sci USA* 2002; 99: 2124-2128

S- Editor Liu Y L- Editor Ma JY E- Editor Lu W