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RAPID COMMUNICATION

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

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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.

Key words: Hypersplenism; Macrophage; cDNA microarray

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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 H_2O 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℃ for 6 h. Fluorescent probe mixtures were denatured at 95℃ for 5 min, and the denatured probe mixtures were applied onto the pre-hybridized chip under a cover glass. Chips were hybridized at 42℃ for 16-18 h. The hybridized chips were then washed at 60℃ 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.

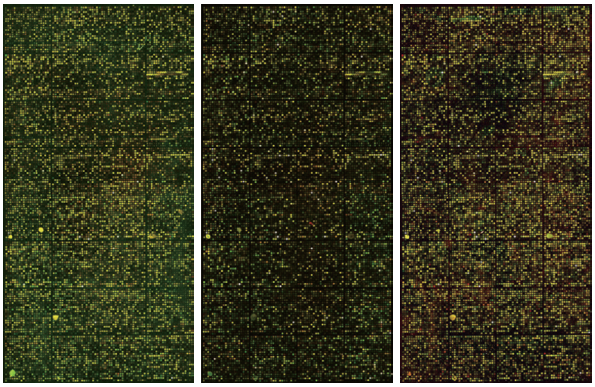

Figure 1 Scanning results of hybridized signals on gene chip. Color of spots in image: high expression (red), low expression (green) and no change in expression (yellow).

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.

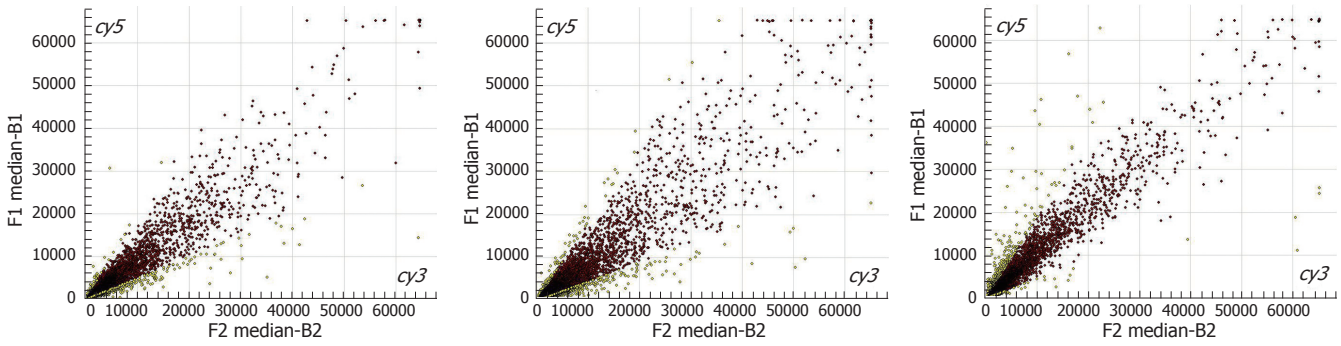

Figure 2 Scatter plots of hybridized signals on gene chip.

Table 1 Differentially expressed genes up-regulated in macrophages of portal hypertensive spleen

GenBank No.	Gene name and description	Average ratio
AF189009	UBQLN2, ubiquilin 2	2.117
BX537509	NET1, neuroepithelial cell transforming gene 1	2.270
NM_004830	CRSP3, cofactor required for Sp1 transcriptional activation	2.334
AF025771	ZNF189, zinc finger protein 189	2.453
AK090727	Homo sapiens cDNA FLJ33408 fis, clone BRACE2010550	2.511
BX537955	TRIM37, tripartite motif-containing 37	2.553
NM_006716	ASK, activator of S phase kinase	2.620
NM_181523	PIK3R1, phosphoinositide-3-kinase, regulatory subunit 1	2.755
XM_376537	Homo sapiens BCL2-associated transcription factor 1 (BCLAF1), mRNA	2.993
NM_004416	DTX1, deltex homolog 1 (Drosophila)	3.553

Table 2 Differentially expressed genes down-regulated in macrophages of portal hypertensive spleen

GenBank No.	Gene name and description	Average ratio
BC068441	IL1RN, interleukin 1 receptor antagonist	0.179
NM_014909	KIAA1036	0.268
BU732296	Homo sapiens cDNA clone UI-E-CI1-afo-e-04-0-UI 3', mRNA sequence	0.275
AK092248	Homo sapiens cDNA FLJ34929 fis, clone NT2RP7004728	0.293
NM_006254	PRKCD, protein kinase C, delta	0.298
BX648172	OAZ2, ornithine decarboxylase antizyme 2	0.302
BM542499	Homo sapiens cDNA clone IMAGE:5521023 5', mRNA sequence	0.311
BF240734	Homo sapiens cDNA clone IMAGE:4091885 5', mRNA sequence	0.324
NM_003902	FUBP1, far upstream element (FUSE) binding protein 1	0.338
BM993772	Homo sapiens cDNA clone IMAGE:5869020 3', mRNA sequence	0.344
NM_005373	MPL, myeloproliferative leukemia virus oncogene	0.359
BX647757	SCML1, sex comb on midleg-like 1 (Drosophila)	0.370
NM_005781	ACK1, activated Cdc42-associated kinase 1	0.381
AL832249	Homo sapiens mRNA; cDNA DKFZp686P1077	0.390
AK092130	LOC285378, hypothetical protein LOC285378	0.406
AF466367	Homo sapiens clone KU011197 unknown mRNA	0.434
NM_001067	TOP2A, topoisomerase (DNA) Ⅱ alpha	0.437
AF051151	TLR5, toll-like receptor 5	0.452

Differentially expressed genes

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 macrophges of portal hypertensive spleen are listed in Table 1 and 18 differentially expressed genes that were down-regulated are demonstrated in Table 2.

DISCUSSION

Since the microarray analysis was first reported by Schena[6] 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.

In order to obtain enough amounts of total RNA and eliminate individual variation, the total RNA of Mφ extracted 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 ionic channel and transport protein, cyclin, cytoskeleton, cell receptor, cell signal conduct, metabolism, immune, and so on.

PRKCD encoding protein kinase C delta[7] was found down-regulated in the Mφ 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]. Those findings indicate that PRKCD has a close relationship with the function of human monocytes (including Mφ). 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 *et al*[11] 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 invoved 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 Mφ 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 Mφ 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 Mφ 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 *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 Mφ of portal hypertensive spleen, the up-regulation of ASK may lead to the activity enhancement (including phagocytosis) of Mφ, 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 Mφ of portal hypertensive spleen, indicating that more insulin existed and the glycometabolism was enhanced in Mφ. 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 Mφ in spleen. However, the possible molecular mechanisms remain undiscovered.

Many differentially expressed genes of Mφ 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.

COMMENTS

Background

The destruction of hemocytes by macrophage of spleen plays an important role in the development of hypersplenism in portal hypertension. The authors have proved that phagocytosis of Mφ is augmented in hypersplenism in portal hypertension; however, the specific mechanisms are not clear.

Research frontiers

The functions of macrophage are focused in the investigation of pathogenesis of hypersplenism in portal hypertension.

Innovations and breakthroughs

This study based on cDNA microarray has screened differentially expressed genes of macrophages between normal spleen and portal hypertensive spleen, which may provide a new idea in studying the pathogenesis of hypersplenism in portal hypertension.

Applications

Small interfering RNAs or other techniques may alter the expression of the differentially expressed genes, and may be used in treatment of hypersplenism in portal hypertension.

Peer review

The authors identified the difference in gene expression of Mφ between normal spleen and portal hypertensive spleen using cDNA microarrays, found that 121 genes were differentially expressed in all three chips, including 21 up-regulated known genes and 73 down-regulated known genes. The differentially expressed genes were related to the ionic channel and transport protein, cyclin, cytoskeleton, cell receptor, cell signal conduct, metabolism, immune, and so on. These genes might be also related to the hypersplenism in portal hypertension. These differentially expressed genes may provide some clues for further studies of hypersplenism in portal hypertension.

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