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膽道閉鎖嬰兒及輪狀病毒感染誘發肝外膽管阻塞鼷鼠之基因表現(1/3)

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中文摘要：

膽道閉鎖症（BA）是一種肝外膽管及肝內膽管呈漸進性、發炎性的損傷，最終導致肝硬化及肝衰竭。這種動態的發炎壞死的膽管病變其致病機轉不明。利用基因微陣列（DNA microarray）是解開膽管閉鎖症所牽涉複雜的基因調控網路一種有力的方法。本研究以膽道閉鎖嬰兒在接受肝臟移植時作全肝切除所取下之肝組織作分析並以活體捐肝手術時，來自捐贈者之正常肝組織作對照組，以了解膽道閉鎖嬰兒其肝組織中生理相關之基因表現之輪廓。我們所用的基因微陣列法是由台大基因體醫學中心所發展的人類寡核苷晶片（含 14208 基因產物）來作分析。作數據過濾及群集分析後我們發現與正常肝組織比較，BA 嬰兒肝組織中主要有與免疫及發炎作用相關之基因過度表現。其中轉譯產物之訊號強度在 2 倍以上的相關基因有 chemokine（c-c motif)ligand 2，osteopontin，cyclin B2，腫瘤壞死因子受器 superfamily and member 10a。其中 osteopontin 係一種細胞外基質蛋白，有類似細胞素之功能且且為細胞性免疫之調節者。在 BA 嬰兒肝組織中也有一些基因其表現顯著低於正常肝組織，包括與 hydrolase 活性、免疫反應或細胞素與其受體互動及 B 細胞調控之免疫性有關之基因。其中 rynodine receptor 1 與 B 細胞生長及 B 細胞調控之免疫性有關。這些初步結果顯示 osteopontin 之過度表現（或類似幫助者 T 細胞第一型細胞素反應）可能在 BA 的致病機轉中扮演某種角色，我們仍繼續對 BA 早期之肝組織及其他新生兒膽汁滯流症作分析，以了解 BA 之分子致病機轉。

關鍵語：基因表現，膽道閉鎖，基因微陣列，發炎作用。

SUMMARY

Biliary atresia (BA) is a progressive, destructive and inflammatory process of extra- and inrahepatic bile ducts resulting in liver cirrhosis or failure. The pathogenesis underlying this dynamic necro-inflammmatory destructive cholangiopathy remains unknown. DNA microarray is potentially able to delineate the complex gene regulation networks involved in the genesis of BA. To understand the physiologically relevant expression profiles of genes in livers of BA , two samples of liver taken by total hepatectomy from BA infants receiving liver transplantation and two nondiseased liver tissue were studied by using an oligonucleotide based human known gene chips containing 14208 gene products. By data filtering, we have identified those genes that were differentially expressed with a > 2 fold changes in BA infants compared with non-diseased control. Further cluster analysis showed a predominant activation of immunity/inflammation genes within the liver of infants with BA. Those with two fold increase or above in the signal intensity of transcripts including genes encoding chemokine (C-C motif) ligand 2, osteopontin, cyclin B2, tumor necrosis factor receptor superfamily and member 10a. Among them, osteopontin is an extracellular matrix protein with important cytokine-like functions (a regulator of cell-mediated [Th-1] immunity). Some genes with known function displaying lower signal intensity in BA than in healthy controls were found and relevant to hydrolase activity, immune response or cytokine-cytokine receptor interaction and B cell growth and B cell-mediated immunity(rynodine receptor 1). These preliminary results suggest that overexpression of osteopontin, namely, the Th-1-like cytokine response, is involved in disease pathogenesis of biliary atresia. Further studies including microarrays of cDNA from livers of infants with early stage BA and from diseased control with neonatal intrahepatic cholestasis are currently under investigation in our laboratory.

Key words: gene expression profile, microarray, biliary atresia

INTRODUCTION

Extra-hepatic biliary atresia (BA) is the leading cause of liver diseases resulting in death in pediatric population.[1] The incidence of BA is higher in Chinese than in Caucasians. BA is the end result of a progressive destructive, inflammatory of extrahepatic as well as intrahepatic bile ducts, resulting in fibrosis and obliteration of the biliary tracts, biliary cirrhosis and finally liver failure requiring liver transplantation.[2] The pathogenesis underlying this dynamic necro-inflammatory destructive cholangitis remains unknown. Evidence to date supports several possible pathogenetic mechanisms for the development of BA.[2] Several studies suggested that biliary epithelial injury of this disease possibly resulting from the interplay between genetic susceptibility and environmental exposure (viruses, toxic agents and metabolic insults) at perinatal period.[1-3] Immunohistochemical studies demonstrated the infiltration of CD4+, CD8+ lymphocytes and natural killer (CD56+) cells predominated in the liver and extrahepatic bile ducts and an increase in CD68+ macrophage infiltration in portal tracts and biliary remnant tissue in BA patients with poor outcome after the portoenterostomy procedure.[5-7] These findings suggested that inflammation involving several kinds of immune cells produce biliary epithelial injury such as pyknosis and necrosis of biliary epithelial cells and also promote hepatic fibrosis and cirrhosis in infants with BA. The mechanisms underlying recruitment of inflammatory cells, immunologic dysregulation, ductular apoptosis and progressive hepatic fibrosis in BA remains unclear. DNA microarrays is potentially able to delineate the complex gene regulation networks involved in the the genesis of BA.

METHODS

Liver tissue was also taken from two BA infants with advanced stages of chronic cholestasis at the time of total hepatectomy for liver transplantation. Liver samples from normal, age-matched infants were not obtained owing to ethical consideration. Liver tissue was also obtained from two healthy adults who were living related donor in liver transplantation and served as controls.

For DNA microarrays, total RNA was extracted using TriReagent (Molecular Research Center, Inc.) according to the manufacturer's protocol. The structural integrity of the isolated RNA was confirmed by electrophoresis of the RNA through a 1.2% gel. Purity of RNA was ascertained by inclusion of samples with an absorbance ratio 260/280 nm >1.8.Each sample was fluorescence-labeled and hybridized to oligonucleotide based human known gene chips (developed in National Taiwan University Center for Genomic Medicine) containing 14208 gene products. (control genes: 768; known genes: 13400). The indirect-labeling method was performed according to J. Hasseman's standard operating procedure for aminoallyl labeling of RNA for microarrays from The Institute For Genomic Research. The hybridization method was performed according to the protocol described by Ideker et al.[8] The hybridized slides were then washed briefly at room temperature with 0.5x SSC and 0.01% SDS to remove the coverslip, followed by three successive room temperature washes with 0.5x SSC, 0.1x SSC, and 0.01x SSC for 2 min for each wash. Slides were dried by centrifugation and immediately scanned. Three independent time-course experiments were performed. The data obtained from experimental repeats were consistent. The slides were scanned with a GenePix 4000 scanner (Axon Instruments, Inc., Foster City, Calif.) as previously described.[9] and analyzed using Genepix Pro 4.0 (Axon Instruments) and a custom designed gene array list file generated by the Bioinformatics and Molecular Analysis Section (BIMAS) at the Center for Information Technology (CIT), NIH. The localized raw expression ratios were calculated following background subtraction, and the expression ratios were normalized using the expression ratios obtained for the control cellular housekeeping genes to obtain a calibrated expression ratio (CalRatio).[10,11] CalRatio data were subsequently uploaded into the Center for Cancer Research Microarray Data Base (mAdb) for spot filtering and for clustering analysis using M. Eisen's Cluster and Tree View software**.** CalRatios were $\log_2$ transformed and clustered using Pearson's correlation coefficient with the hierarchical clustering algorithm and Cluster and TreeView in the mAdb analysis toolset. Then we analyzed the signal intensity for this group of genes by the two-tailed, unpaired Student's t test to identify genes differentially expressed in liver of children with biliary atresia or normal healthy donor liver.

RESULTS

Data filtering and cluster analysis identified those transcripts with significant differences in mean signal intensity between BA patients and healthy controls and generated a profile of genes overexpressed or underexpressed in livers of biliary atresia.. We identified those genes uniquely expressed in biliary atresia compared with healthy controls. The reproducibility of the signal intensity was confirmed for two genes by RT-PCR. If we consider the genes with increased signal intensity in BA, a major biological process can be identified in this disease, i.e. immunity and inflammation. The relevant genes included chemokine (C-C motif) ligand 2, and genes encoding osteopontin, cyclin B2, tumor necrosis factor receptor superfamily and member 10a. Among them, the 3.4 fold increase in the signal intensity for osteopontin was noted. This is an extracellular matrix cell adhesion protein which is also a novel substrate for MMP-3 and MMP-7. MMP-cleaved osteopontin has increased activities in cell migration, cell survival, and most notably, cytokine-like functions such as a central regulator for the T-helper 1 (Th-1) commitment of lymphocytes in vivo Thus, an activation of a cell-mediated immune response might occur in affected livers. On the other hand, several genes with known function displaying lower signal intensity in BA than in healthy controls are protein tyrosine phosphatase receptor type A (relevant to hydrolase activity and inflammatory response), complement component 9 (relevant to cytolysis and immune response), interleukin 2 receptor, gamma (relevant to cytokine-cytokine receptor interaction) and rynodine receptor 1 (relevant to B cell differentiation, proliferation and B cell-mediated immunity). The significance of the lower expression of several immunity/inflammation genes in BA such as a suppressed B lymphocyte functions within the liver in late stage of BA needs further studies.

DISCUSSION

The results revealed a transcriptional profile in the liver of infants with biliary atresia with coordinated activation or suppression of specific genes which differentiates this disease from normal healthy individuals. An increased expression of osteopontin at the time when biliary cirrhosis develops in infants with biliary atresia suggests that Th-1 like cytokines play a significant role in the pathogenesis of biliary atresia. Direct comparison in gene expression with age-matched healthy infants was not possible due to ethical reasons in obtaining liver biopsy samples from healthy, age-matched infants. It has been shown that T-helper phenotype in circulating mononuclear cells can be skewed toward a Th-2 phenotype at birth, but this functional commitment may depend directly on a timely exposure to antigens.[12,13]

Bezerra et al.[14] analyzed a large-scale gene expression in the livers of infants with BA or neonatal intrahepatic cholestasis. They found a predominant and coordinated activation of immunity/inflammation genes within the livers of infants with BA. Most of the genes showed differential lymphocyte function, with activation of oeteopontin, a regulator of cell-mediated (T-helper 1) immunity in T-helper lymphocytes and suppression of immunoglobulin genes in early stages of disease.

Our preliminary results, therefore, add further support to the view that a dominant proinflammatory differentiation of lymphocytes in affected livers may have a key role in the pathogenesis of BA. Further studies including microarrays of cDNA from livers of infants with early stage BA and from diseased control with neonatal intrahepatic cholestasis are currently under investigation in our laboratory. In addition, the disease model of rotavirus-induced hepatobiliary injury and obstruction in young mice may be a promising experimental alternative in the future.[15,16]

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