Abstract

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Project Title : Whole genome gene expression study, pre treatment, in PBMC of chronic hepatitis B patients that respond and not respond to PEG-Interferon alfa-2b

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The sustained virological responder (SVR) to pegylated interferon (PegIFN) can be achieved in only one-third of patients with chronic hepatitis B (CHB). In this study, we try to identify host biomarker that could be used for differentiating SVR from non-responders (NR). First, we investigated global gene expression patterns in peripheral blood mononuclear cells (PBMC) from 14 CHB patients (SVR=7, NR=7) received PegIFNα-2b by the Illumina Sentrix Humanref-8 v2 BeadChips at pre-treatment and 24 weeks after treatment. The expression of the selected genes was validated by real-time RT-PCR. In summary, our study indicated that the global expression signature of PBMC in pre-treatment and post-treatment can be potential biomarkers for predicting treatment outcome. While specific gene expression *in vivo* gave variable result and have limited use in prediction, the expression change after treatment with PegIFN *ex vivo* may be a better prediction tool. Second, we also investigated serum proteomic profile from 9 SVR and 10 NR at pre-treatment and 24 weeks after treatment by 2-DE and MS/MS analysis. 7 differentially expressed proteins were discovered including CD5 antigen-like precursor, α-2-HS-glycoprotein and Apolipoprotein A-I which were potential markers in the serum that can be used to predict response to PegIFN. Lastly, we were interested in elucidating the complex role of cytokines in susceptibility to CHB; therefore, we investigated association study of 21 SNPs from 13 cytokine and cytokine receptor genes using LIFECODES Cytokine SNP Typing kit. Interestingly, the TCC (-1098/-590/-33) haplotype frequency of *IL-4* showed a positive association with CHB as a protective haplotype (OR=0.53, 95%CI=0.32-0.85, p=0.005). These results suggest that polymorphisms in some cytokine genes particularly the Th2 cytokine influence persistent HBV infection.

Keywords Chronic Hepatitis B, Gene expression profiles, Pegylated interferon, Proteomics

Gene Expression Signature and the Prediction of Responses to Pegylated Interferon Treatment in Chronic Hepatitis B

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Abstract

Hepatitis B infection is a major cause of liver disease. Approximately 2 billion people worldwide and 400 million of them remain chronically infected. The sustained virological responder (SVR) to pegylated interferon (PegIFN) can be achieved in only one-third of patients with chronic hepatitis B (CHB). In this study, we investigated whether gene expression patterns in peripheral blood mononuclear cells (PBMC) could be used for differentiating sustained virological responders from non-responders. Fourteen untreated CHB patients who meet the inclusion criteria received PegIFNα-2b for 48 weeks. The gene expression profiles in PBMC samples of CHB patients were analyzed by the Illumina Sentrix Humanref-8 v2 BeadChips. There were 146 genes that expressed higher in the SVR group and 81 genes that expressed higher in the NR group. The enriched biological pathway of 146 genes analyzed by IPA software included Granzyme B signaling, apoptosis signaling, CTL mediated apoptosis of target cells. Among these genes that expressed higher in the SVR group, IFNA1 was the only gene in the IFN signaling. The enrich pathway of 81 genes were ICOS-ICOSL signaling in T helper cells and IL-17 signaling. The expression of the selected genes was validated by real-time RT-PCR. The expression of 5 genes (IFNAR1, ATXN2, GLS, TTC15 and ZNF331) were significantly differentially expressed between SVRs and NRs ($p<0.05$). We further explored global gene expression profile in these patients at 24 weeks after treatment with PegIFNα-2b compared to baseline (SVR=7, NR=7). We found 71 genes that express significantly different between the responder and non-responder groups. Among these genes, 39 out of 71 genes changed their expression significantly higher in SVR than in NR, while 32 out of 71 genes changed their expression significantly higher in NR than in SVR. Using the gene expression changing signature of 71 genes, treatment outcome can be predicted correctly. Using STRING

and DAVID to find relationships among these genes, we found gene clusters associating with mRNA processing and splicing, JAK-STAT signalling pathway, regulation of cell proliferation, interferon binding, and intracellular signalling cascade. Five selected genes were validated in PBMC after treatment at 12 weeks, 24 weeks and 36 weeks compared to pre-treatment by real time RT-PCR (SVR=13, NR=25). The expression of each selected genes did not significantly differ between SVR and NR groups but have a same trend at 24 weeks as in microarray. Lastly, we performed the *ex vivo* study, PBMCs isolated from blood samples of SVR and NR groups at pre-treatment were cultured with or without 200 U/ml of PegIFNα-2b for 6 hours and the change of gene expression were measured by real-time RT-PCR (SVR=4, NR=3). Interestingly, we found that the expression of 2 genes were significantly different between SVR and NR groups (*ZNF175*; p=0.0001 and *IFNAR1*; p=0.01). However, this finding needs further validation in larger sample size. In summary, our study indicated that the global expression signature of PBMC in pre-treatment and post-treatment can be potential biomarkers for predicting treatment outcome. While specific gene expression *in vivo* gave variable result and have limited use in prediction, the expression change after treatment with PegIFN *ex vivo* may be a better prediction tool.

Keywords: Chronic Hepatitis B, Gene expression profiles, Response to Pegylated interferon