Interferon Alfa Regulated Gene Expression in Patients Initiating Interferon Treatment for Chronic Hepatitis C

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Interferon alfa (IFN-α) is an approved therapeutic agent for chronic hepatitis C. To directly characterize the effects of IFN-α in humans, we used microarrays to profile gene expression in peripheral blood mononuclear cells (PBMCs) from hepatitis C patients treated with IFN-α. Seven patients were studied using two strategies: (1) *in vivo*: PBMCs were collected immediately before the first dose of IFN-α, and 3 and 6 hours after the dose; (2) *ex vivo*: PBMCs that were collected before the first IFN-α dose were incubated with IFN-α for 3 and 6 hours. The microarray datasets were analyzed with significance analysis of microarrays (SAM) to identify genes regulated by IFN-α. We identified 516 named genes up-regulated at least 2-fold, at a false discovery rate (FDR) of less than 1%. *In vivo* and *ex vivo* studies generated similar results. No genes were identified as regulated differently between these 2 experimental conditions. The up-regulated genes belonged to a broad range of functional pathways and included multiple genes thought to be involved in the direct antiviral effect of IFN-α. Of particular interest, 88 genes directly relating to functions of immune cells were up-regulated, including genes involved in antigen processing and presentation, T-cell activation, lymphocyte trafficking, and effector functions, suggesting that IFN-α up-regulates multiple genes involving different aspects of immune responses to enhance immunity against hepatitis C virus. In conclusion, IFN-α–inducible genes can be identified in human PBMCs *in vivo* as well as *ex vivo*. Signature changes associated with different treatment outcomes may be found among these genes. (HEPATOLOGY 2003;37:610-621.)

Interferon alfa (IFN-α) alone or in combination with ribavirin are the only Food and Drug Administration–approved treatments for chronic hepatitis C. The treatment has a low sustained virologic response rate[1-3] and often is accompanied by significant side effects.[2] The mechanisms for the anti–hepatitis C virus (HCV) effects of IFN-α are largely unknown. They are believed to include IFN-α–induced changes in hepatocyte gene expression that directly suppress HCV replication, and the modulation of immune cell functions that enhances antiviral immunity. *In vitro* studies with tissue culture cells revealed that IFN-α up-regulates expression of genes encoding a wide variety of proteins, some of which have been associated with direct or indirect antiviral effects.[4] However, the overall *in vivo* effect of IFN-α on gene expression in patients receiving IFN-α treatment is characterized poorly.

Recently developed microarray technology[5] enables simultaneous monitoring of expression of thousands of host genes, providing an opportunity to study complex signal transduction systems at the genomic level. To extend our understanding of the antiviral mechanism of IFN-α and to eventually identify IFN-α–regulated genes directly associated with treatment outcome, we have used microarray analysis to characterize expression of genes responding to IFN-α in chronic hepatitis C patients starting IFN-α treatment. Our first goal was to assess the general pattern of gene expression induced *in vivo* by IFN-α treatment. To examine whether significant differences existed in the pattern of gene expression induced by IFN-α *in vivo* and *ex vivo*, we have conducted experiments under both conditions.

Abbreviations: IFN, interferon; HCV, hepatitis C virus; mRNA, messenger RNA; SAM, significance analysis for microarray; PBMC, peripheral blood mononuclear cell; cDNA, complementary DNA; FDR, false discovery rate; ISG, IFN-stimulated genes.

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Table 1. Microarray Analysis of Chronic Hepatitis C Patients Treated With IFN-α

			Liver Pathology			Number of Microarrays			
						In Vivo		Ex Vivo	
Patient	Age	HCV Genotype	Grade	Stage	Virologic Response	3 h	6 h	3 h	6 h
P1	55	3a	3	1-2	Sustained responder	1	2	ND	2
P2	48	3a	4	1-2	Relapser	1	ND	1	ND
P3	58	1a	3	3	Nonresponder	1	ND	1	ND
P4	65	3a	3	2	Nonresponder	1	ND	1	ND
P5	55	1a	3	3	Nonresponder	1	3	1	1
P6	56	1a	2	2-3	Nonresponder	1	3	2	2
P7	45	3a	2	2	(Not applicable)	1	1	1	2

NOTE. The virologic response to the treatment was determined by standard qualitative HCV-RNA test (Amplicor, Roche Laboratories, with a sensitivity of 50 IU/mL). Nonresponder is defined as a positive serum HCV RNA at the end of 6-month treatment. Relapser is defined as a negative serum HCV RNA at the end of 6-month treatment and a positive serum HCV RNA 6 months after the treatment. Sustained responder is defined as a negative serum HCV RNA both at the end of 6-month treatment and 6 months after the treatment. Patient P7 stopped treatment after the first dose of IFN-α.

Abbreviation: ND, not done.

Most prior microarray studies relied on fold-change of the messenger RNA (mRNA) level to define up-regulated or down-regulated genes, or relied on cluster analysis[6] to identify coherent patterns of multiple gene expression. Although these analytic approaches may reveal intriguing information regarding different biologic processes, they provided little information about statistical significance. Specifically, it has been shown that the approach of classifying genes as significantly changed based solely on fold-change criteria may result in an unacceptably high rate of false positives.[7] In the current study, we use significance analysis of microarrays (SAM)[7] to search for genes regulated by IFN-α *in vivo* or *ex vivo*, and genes differentially respond to IFN-α under different conditions. These studies generate a comprehensive picture of IFN-α–induced genes in humans, which forms the basis of this report.

Patients and Methods

Patients and Blood Samples. Seven patients (Table 1) starting interferon alfa-2b/ribavirin (Schering, Kenilworth, NJ) combination therapy for chronic hepatitis C were enrolled. The study protocol was approved by the Administrative Panel on Human Subjects in Medical Research of Stanford University. Informed consent was obtained from each subject. Peripheral blood mononuclear cells (PBMCs) were prepared from venous blood by using a Cell Preparation Tube with sodium citrate (BD Vacutainer Systems, Franklin Lakes, NJ), which is based on Ficoll gradient centrifugation.

In Vivo *and* Ex Vivo *Studies.* For *in vivo* study, blood samples were collected from the patients immediately before the first dose of IFN-α, and 3 and 6 hours after the injection, before the first ribavirin dose. For *ex vivo* study, aliquots of PBMCs isolated from the pre–IFN-α blood samples were incubated at 37°C in RPMI 1640 medium, supplemented with 10% fetal calf serum at 10^6 cell/mL, with or without 200 U/mL of IFN-α–2b, for 3 and 6 hours.

Preparation of Fluorescence-Labeled Targets and Complementary DNA Microarray Hybridization. Total RNA was extracted from PBMCs by using Trizol LS Reagent (Life Technologies, Grand Island, NY). mRNA was amplified by using a previously described strategy.[8] In brief, 3 μg of total RNA was used to synthesize double-strand complementary DNA (cDNA) that contained a promoter for T7 RNA polymerase. Amplified antisense RNA was synthesized by *in vitro* transcription of the cDNA templates by using the T7 Megascript kit (Ambion, Austin, TX). The antisense RNA was labeled by Cy3–deoxyuridine triphosphate or Cy5–deoxyuridine triphosphate (Amersham, England, UK), using Superscript II (Life Technologies) with random hexamer primer. For the *in vivo* study, the antisense RNA derived from PBMCs of the pre- and postinjection blood samples were labeled with Cy3 and Cy5, respectively. For the *ex vivo* study, the antisense RNA derived from PBMCs incubated with and without IFN-α were labeled with Cy5 and Cy3, respectively. The Cy3- and Cy5-labeled targets were mixed and hybridized to cDNA microarrays as previously described.[8,9] Based on the availability of blood samples, duplicate or triplicate microarray hybridizations were performed for some subjects (Table 1). In each of the repetitive experiments, Cy3/Cy5-labeled targets were prepared by independent cDNA synthesis, antisense RNA amplification, and labeling reactions. A total of 30 microarray datasets were generated and analyzed. The microarrays were obtained from Stanford Functional Genomics Facility (Stanford, CA). Each microarray contained 39,552 array elements corresponding to 38,432 human sequence–verified genes.

Signal Detection and Processing. The fluorescence signal on microarrays was acquired by using a GenePix 4000b microarray scanner (Axon Instruments, Foster City, CA). The scanned images were processed by using the GenePix Pro 3.0 software (Axon Instruments). The data files were entered into the Stanford Microarray Database.[10] The red (Cy5) signal on all the array elements was normalized so that the median Cy5/Cy3 ratio of all nonflagged elements on each array was equal to 1. A filter was set to select array elements with a regression correlation of R greater than 0.6.

Significance Analysis of Microarray Data. The normalized and filtered Cy5/Cy3 ratios of all microarray elements were downloaded from the Stanford Microarray Database in 4 groups, resulting in the following 4 sets of microarray data: *ex vivo* 3-hour, *ex vivo* 6-hour, *in vivo* 3-hour, and *in vivo* 6-hour (Table 1), which were analyzed with SAM.[7] Duplicate and triplicate arrays derived from the same blood samples, as well as arrays derived from different subjects in the same group, were treated equally as repeated measurements. To identify IFN-α–regulated genes within each group, 1-class SAM analysis was performed. This analysis generated a list of genes with an average Cy5/Cy3 ratio significantly different from 1.0, along with an estimate (at 90% confidence) of the percentage of such genes identified by chance, or the false discovery rate (FDR), which is based on permutations of repeated measurements.[7] FDR can be adjusted by setting a threshold Δ at different stringencies, resulting in different numbers of genes whose change in expression were classified as significant. To identify genes regulated differently between two groups, 2-class SAM analysis was performed, which generated a list of genes with significantly different average Cy5/Cy3 ratios accompanied with an estimate (at 90% confidence) of FDR.

Annotation of Gene Functions. A list of all IFN-α–inducible genes identified by 1-class SAM analysis was first compiled. The criteria for a gene to be included were: identified in at least 1 of the 4 experimental groups, FDR less than 1%, and the average fold-change greater than 2. A total of 904 genes, including 516 named genes and 388 hypothetical proteins, expressed sequence tags, and other unnamed human cDNA clones met these criteria. The named genes were first analyzed by using the proprietary program SAGA (Eli Lilly and Company, Indianapolis, IN), which automatically classified the genes into known function/pathway groups. Genes that remained unclassified after SAGA analysis were assigned manually a functional classification based on information retrieved from Stanford Online Universal Resource for Clones and expressed sequence tags (SOURCE) (http://genome-www5.stanford.edu/cgi-bin/SMD/source/sourceSearch).

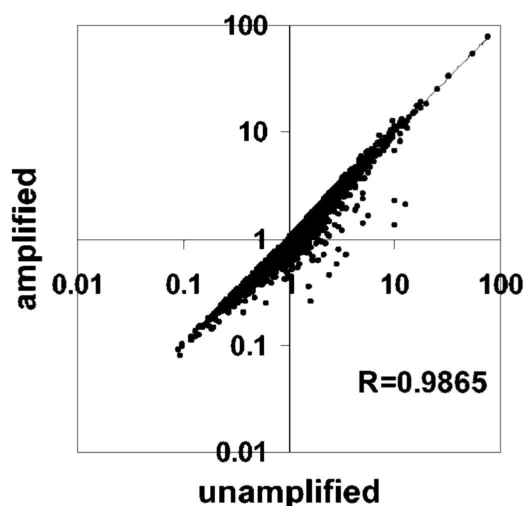

Fig. 1. Comparison of microarray datasets derived from amplified or unamplified mRNA. CD8 T-cell clone 78 (unpublished result) specific for the HCV $NS3_{1406-1415}$ epitope was stimulated with the cognate peptide or a control peptide for 6 hours, followed by isolation of total RNA and then mRNA. Three micrograms of unamplified mRNA from the $NS3_{1406-1415}$ peptide- or control peptide–stimulated cells were labeled with Cy5 or Cy3, respectively, mixed, and hybridized to a microarray. Three micrograms of total RNA from the 2 cell cultures were amplified as described in the Patients and Methods section and then labeled with Cy5 or Cy3, respectively, mixed, and hybridized to another microarray. The scatter plot shows the Cy5/Cy3 ratios of a total of 7,001 array elements that had passed filtering and were present in both microarray datasets.

Results and Discussion

Verification of RNA Amplification Procedure. Most of the published cDNA microarray studies use fluorescence-labeled targets directly prepared from purified mRNA.[5] To isolate enough mRNA from PBMCs for direct labeling, approximately 200 mL of whole blood is needed for a single microarray experiment, which would not be feasible for many clinical studies. Therefore, we used an amplification procedure based on antisense RNA synthesis by *in vitro* transcription of cDNA templates prepared from total RNA.[8] To verify the fidelity of the amplification, we used a CD8 T-cell clone activated with its cognate peptide. Two microarray experiments were performed to profile changes in gene expression induced by T-cell activation, using either unamplified mRNA, or amplified antisense RNA derived from total RNA, respectively. A scatter plot of Cy5/Cy3 ratios for genes from both microarrays shows that the 2 datasets are highly correlated (Fig. 1), indicating that the amplification procedure did not distort the general pattern of gene expression detected by microarray assay.

IFN-α–Induced Gene Expression Under Different Experimental Conditions. The numbers of IFN-α–regulated genes identified by 1-class SAM analysis for each experimental condition are shown in Fig. 2. At FDR less

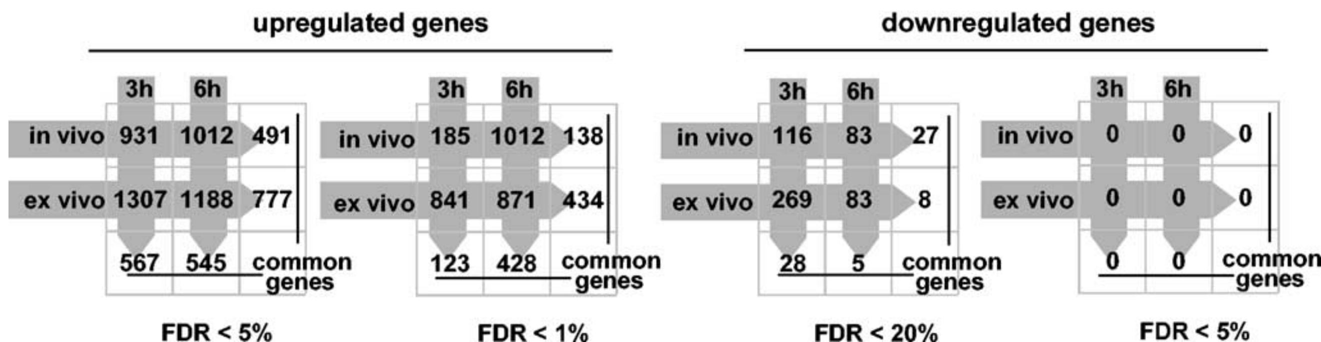

Fig. 2. Number of IFN-α–regulated genes under different experimental conditions, identified by 1-class SAM analysis.

than 5%, 931 to 1,307 genes were up-regulated under the 4 experimental conditions. At FDR less than 1%, a more stringent criterion, the number of up-regulated genes remained unchanged in the 6-hour *in vivo* group but was reduced by 80% in the 3-hour *in vivo* group, and declined by 28% to 36% in the 2 *ex vivo* groups. The number of down-regulated genes was much smaller and was accompanied by a much higher FDR. This reflects the fact that even after the transcription of a specific gene stops completely, its mRNA still persists for a period depending on its degradation rate. Therefore, any assay system based on the net amount of mRNA is likely to be less sensitive at detecting down-regulated genes compared with up-regulated ones.

The average fold-change in mRNA level was determined for all the up-regulated genes identified at FDR less than 1% and their distribution is shown in Fig. 3. In each group, between one third and two thirds of the genes changed their expression level by more than 2-fold after treatment with IFN-α.

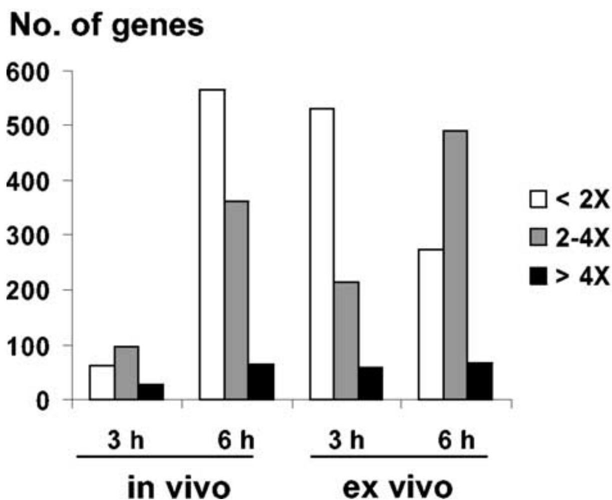

Fig. 3. Distribution of fold-change value in mRNA levels of genes up-regulated by IFN-α under 4 experimental conditions. Included genes were identified by 1-class SAM analysis at FDR less than 1%. □, $<2\times$; ▩, 2-4×; ■, $>4\times$.

We next compared the different gene lists to identify genes commonly regulated under different experimental conditions. As shown in Fig. 2, when FDR was set at less than 5%, the *in vivo* and *ex vivo* groups shared approximately half of their up-regulated genes. This indicates a broad similarity in up-regulated genes between *in vivo* and *ex vivo* conditions. The similarity also existed between the 3- and 6-hour groups. When FDR was reduced to less than 1%, the number of up-regulated genes in the 3-hour *in vivo* group decreased from 931 to 185, whereas those in the other 3 groups changed much less or remained unchanged. However, more than two thirds of the 185 genes up-regulated in the 3-hour *in vivo* group were still shared with other groups. Six hours after IFN-α injection, the number of up-regulated genes in the *in vivo* group surpassed that of the *ex vivo* group. This suggested that under *in vivo* conditions, the majority of IFN-α–stimulated genes had initiated up-regulation by 3 hours after IFN-α injection but the change was less significant at this time point. Therefore, when a more stringent criterion (FDR $<$ 1%) was applied, they were eliminated from the significant gene list. By 6 hours, this difference between *in vivo* and *ex vivo* conditions largely disappeared. The number of down-regulated genes also showed a delayed response under *in vivo* conditions.

The delay in IFN-α–induced *in vivo* changes of cellular mRNA levels is concurrent with the delayed absorption of IFN-α after subcutaneous injection. Although IFN-α was absorbed relatively slowly after subcutaneous administration with an absorption half-life of 5.5 hours,[11] it is known that 6 to 8 hours after administration of IFN-α, the serum HCV load starts to decline.[12] With a virion half-life estimated to be 3 hours,[13] the IFN-α–induced changes in host cell gene expression directly responsible for the suppression of HCV replication must have occurred much earlier than the decline of serum viral load. This is in agreement with our finding that at 3 hours after IFN-α treatment, expression of over 1,000 genes already had changed. This suggests that genes regulated as

early as 3 hours after IFN-α treatment were likely to include those involved in the direct suppression of HCV.

The 1-class SAM analysis provides information on genes up-regulated in each experimental group, but does not provide any information on genes that are differentially regulated between different groups. To identify genes that are differentially regulated under different experimental conditions, we used SAM (2-class unpaired sample analysis) to search for genes with different average Cy5/Cy3 ratios between *in vivo* and *ex vivo* experiments, as well as between the 3- and 6-hour experiments. No such genes were identified with an FDR smaller than 20% (data not shown). These analyses suggest that the overall responses to IFN-α under the *in vivo* and *ex vivo* conditions are similar, and that the up-regulated mRNA levels of many genes can be detected as early as 3 hours after treatment and maintained to the 6-hour time point.

The *in vivo* strategy provides an actual picture of gene expression in IFN-α–treated patients. However, the observed response may be affected by the heterogeneity in pharmacokinetics of IFN-α between patients or even variation in injection deposition. It has been reported that the maximum serum concentration after injection of IFN-α was 18 to 116 U/mL, which occurred 3 to 12 hours after administration.[11] On the other hand, although the *ex vivo* strategy ensures better control over the concentration of IFN-α, the PBMCs are subjected to extra manipulations and are isolated from their natural environment. Despite these differences, the lists of significant genes generated under these 2 conditions exhibit substantial similarities, both in the total number as well as in the identity of genes included. Not a single gene was identified with a significantly different response after *in vivo* or *ex vivo* treatment. Considering the complicated clinical and logistic issues involved with the *in vivo* study, the *ex vivo* analysis may be an acceptable alternative in future studies for identifying IFN-α–regulated genes associated with different treatment outcomes.

Annotation of Gene Functions. A total of 904 genes, including 516 named genes, were identified in at least 1 of the 4 experimental groups to be up-regulated by IFN-α by at least 2-fold. By using SAGA and SOURCE (see the Patients and Methods section) these named genes were classified into known function/pathway groups. These analyses resulted in 430 genes with at least one functional assignment and 86 unclassified genes. Table 2 shows the complete list of these genes grouped by their functional assignments. Genes with multiple functions were listed under each functional pathway assigned to them, therefore the total number listed in Table 2 exceeds the number of individual genes. The numbers of IFN-α–regulated genes in all the functional pathways are summarized in Table 3.

Previous microarray analyses of 2 human and 1 murine cell line treated with type I and type II IFNs have resulted in a database of IFN-stimulated genes (ISG).[14,15] By choosing genes with increased expression of at least 1.5-fold (for IFN-β) or 2-fold (for IFN-α), 335 genes were identified. In the current study, we have compiled a list of 904 named and unnamed genes as ISG, with an FDR smaller than 1% and a minimum fold change of 2. We applied the arbitrary 2-fold cut-off value to be consistent with common practice in the field of microarray studies. However, this arbitrary cut-off value resulted in exclusion of approximately 50% of the genes that were otherwise significantly up-regulated according to SAM. It is important to note that these excluded genes may still be biologically relevant. A previous study has shown that a change in mRNA level as small as 1.4-fold can be associated with biologic effect.[16] A complete list of all genes with statistically significant changes in response to IFN-α is available at http://www.stanford.edu/~r2llabc. A comparison of Table 2 with the on-line ISG database[15] showed that approximately 60% of the previously reported ISG overlap with genes identified in this study. Therefore, our results have expanded current knowledge of IFN actions by identifying a large number of statistically defined novel ISG, as well as by confirming, in human PBMCs, the ISG previously identified in human or animal cell lines.

IFN-α–Regulated Genes in Different Functional Groups. Among the functional categories listed in Table 3, we have identified 88 genes directly related to immune system functions. Pertinent to humoral immunity, multiple immunoglobulin genes were up-regulated. Regarding cellular immunity, the up-regulated genes involved almost every component of this system. For example, T cells played a pivotal role in the adaptive immune response against viruses. CD4 (helper) and CD8 (cytotoxic) T cells recognized viral peptide epitopes presented by major histocompatibility class II or I molecules, respectively. In our experiments, both class I and II genes were up-regulated by IFN-α. Genes for multiple proteasome subunits and the transporter 1 protein involved in processing and transporting class I–restricted peptide epitopes[17] were up-regulated as well. In addition, cathepsin L, a lysosomal peptidase involved in the processing of class II–restricted peptide epitopes,[18] was up-regulated. CD86 (B7-2)[19] is an important costimulator for activating naive T cells. Without the costimulating signal, antigen-specific T cells will develop tolerance or die rather than be activated and proliferate on contact with cells presenting viral antigens. In our analysis, CD86 was up-regulated within 6 hours of IFN-α treatment. Selectin L,[20] a chemokine receptor that

Table 2. Genes Up-regulated by IFN-α

GenBank Accession Number	Gene Name/Symbol	Fold Change			
		In Vivo		*Ex Vivo*	
		3 h	6 h	3 h	6 h
Antigen recognition					
R79170	Fc of IgE		3.5		2.7
AA453258	Fc of IgG		2.9		2.7
AA630800	IFI-30		2.3		2.1
AA476918	Ig kappa			2.6	2.7
H73590	1GHM				2.0
Antigen processing and presentation					
W73874	cathepsin L			2.7	2.0
H17513	HSP A1L		2.6		
AA464246	MHC I, C		2.2	2.3	4.4
AA702254	MHC II, DO α		9.0	2.9	
AA669055	MHC II, DQ β1		3.4	2.4	3.7
AA664195	MHC II, DR β1		2.2		3.3
H52245	MHC II, DR β5		2.5	2.2	3.4
H65394	PA28 β		2.6		
R71913	PSMA2		2.1		
AA206497	PSMA4		2.7		
AA181300	PSMB8	2.6	3.7	3.1	2.9
T69304	tapasin		2.5		2.4
AA487429	transporter 1	2.9	3.4	3.2	3.3
AA490855	ZNF173	2.9	2.7	3.2	4.5
Cell-mediated cytotoxicity					
AA490894	A-LAP				2.4
N80692	CD8,α			2.1	3.1
A1200114	granzyme B			2.2	2.6
A1076019	perforin 1			4.1	
H54628	TNF 10	8.1	11.9		
AA166695	TNF 13b		5.5		
AA293570	TNF RSF6		3.9		
Inflammatory response					
W69953	AIF-1		2.2		
AA490894	A-LAP				2.4
AA464711	C3AR			2.8	4.7
T95052	caspase 1		2.5		
H80711	caspase 10			2.3	
AA036881	C-C CKR-1	2.8	4.3	3.1	2.6
H02307	FLAP				2.1
AA425249	FPR1		3.0		
AA479357	fusin				3.2
AA082747	IL 8				2.1
H78386	IL-IR-2				3.4
T71181	ILICIL-IRA	9.4	9.7	12.3	6.0
R47892	LD78			2.1	
AA425102	MCP1 A2			8.4	8.3
AI268937	MCP-2		3.6	2.3	
AA040170	MCP3			10.5	3.8
AA677522	MIP1A			2.2	
AA700005	SI00A12		4.0		
AA086471	SI00A8		2.3		
AA864554	SI00A9		2.2		
AA863403	STAT 3		3.8	3.0	3.9
AA166695	TNF, 13b		5.5		
Other immunity and defense					
AA985421	1-8D		3.6	2.5	
AA464416	1-8U		4.8	2.8	
AA017417	1R20	4.1		3.7	
AA464731	calgizzarin		2.6		2.1
AA436187	CD11b		2.6		2.8
W87269	CD14		2.3		
R22412	CD31		2.0		
R05415	CD48			2.2	
H16746	CD86		2.5	2.6	2.4
AA490216	CKIP-1		2.7		2.0
AA156079	CTSS			2.0	3.9
T51539	D1F15SIA				2.3
H12338	DAP12		2.4	2.1	2.5
N64862	FYB		2.1		
W72748	GBP2	2.8	3.6	3.5	3.1
A1246316	HIMAP2			2.3	
AA969475	IFGR2		2.2		
AA432030	IFI-6-16	2.9	3.7	3.7	6.0
AA504832	IF175	4.6	4.9	4.7	
AA437226	IL10R				2.6
AA279147	IL3RB		2.5		
AA485353	lectin	2.6			
AA227142	LITAF				2.5
AA044166	L-plastin				2.2
AA608576	MAPRE2				2.1
AA400258	MIG-6			2.5	
A1364884	MNDA		4.6		
N71028	MS4A6		5.3		2.3
A1342012	MSR1				2.0
AA872098	NCF-2			2.1	3.1
R38031	peflin				2.2
H52256	properdin P		2.0		
H73484	px19-like		2.1		
H00662	selectin L	2.7	3.4	4.1	6.0
AA927372	SLP76		2.7	2.9	2.4
AA416552	SRPSOX				3.4
AI289185	STAP-1			2.5	
AA083407	TRIM 22	3.2	3.6	4.2	3.9
Differentiation and proliferation					
T40211	ABP125		2.1		
AA453275	ABRI		2.6	2.7	4.6
AA478959	ADPRTL1	2.1	2.3	2.4	2.2
N67778	AML2				2.4
AA004862	ASAP 1				2.0
AA101760	BLMH				2.5
AA485371	BST-2	4.6			
H26183	C/EBP, β		3.0		
T95052	caspase 1		2.5		
AA442092	catenin, β1				2.1
N27179	CD157		2.1		
H70124	CD53			4.5	2.7
R98055	CECR1		3.1	2.4	3.8
AA046523	CEN3		2.9		
AI016999	COMT				2.0
T71991	CREG		2.2		
AA279147	CSF2RB		2.5		
AA086475	cullin 5				2.2
R25725	CYLD1		2.0	2.4	2.8
T51539	D1F15S1A				2.3
R25377	DEK		2.6		
N66144	FYN				2.2
H67342	GST5				3.3
AA490996	IF1 16	3.4	4.5	4.3	3.2
AA419251	IF1TM1	4.1	6.4	7.2	6.3
H78386	IL IR2				3.4
AA431428	inhibin, β		2.2		
W32272	IQ motif				2.4
AA454711	JUNB				2.5
N66278	JUND		2.2		3.2
W72110	keratin 5		3.0		
AA280651	LMO2	3.5	3.4	3.8	4.0
R83836	LYN	2.5	3.9	2.9	3.6
AA608576	MAPRE2				2.1
R52824	MYCN				2.8
H38383	MYD88	3.5	3.6	4.3	5.6
AA669758	NPM1				2.5
N23941	p21			3.0	
AA194811	P2R		2.2		2.2
N63635	pim-1		2.2	3.2	3.4
N25425	RAF 1				2.3
AA205665	SET				2.4
W85832	SRP40				2.0
AA455614	syntenin		2.1		
H51705	TGIF			2.1	2.4
AA633901	TGIFb		2.4		
W47350	TIG3		2.1		2.7
T57791	TLR2		2.8		
AA486085	TMSB10		2.4		2.1
A1299356	TMSB4X			2.1	2.5
AA464245	TROB1		2.0		2.1
AA670215	TSG10		2.4	2.5	2.5
T55399	YES 1	3.0	4.8	4.1	4.9
R38383	ZNF36		3.4		4.0
Cell cycle					
AA278137	B56G			2.1	2.6
AA465166	BM-001			2.3	2.4
AA009465	CDC 42		2.0	2.0	2.8
AA633993	CDC10				2.2
AA676387	CPR2		2.4		2.7
AA486790	cullin 1	2.6	3.1	2.7	2.7
AA599138	cyclin M4		2.2		
AA156796	DKFZP564A2416				2.1
AA419251	IFITM1	4.1	6.4	7.2	6.3
N23941	p21			3.0	
AI015359	PP-1G			2.1	2.4
AA045192	RB1		4.1	2.2	3.2
R76459	septin 6				2.2
Apoptosis					
H94670	14-3-3				2.8
AA490894	A-LAP				2.4
AA971543	APOL3		2.7	2.4	3.3
AI017240	BAG-1	2.5	2.3	4.0	4.9
AA459263	BCL2L5		2.5		
R19628	BIRC2				3.5
AA451935	BIRC5				2.1
AA453766	CASH			2.1	2.2
H80711	caspase 10			2.3	
H10012	CBX4		2.4		
AA490216	CKIP-1		2.7		2.0
AA086475	cullin 5				2.2

Table 2. *Continued*

GenBank Accession Number	Gene Name/Symbol	Fold Change In Vivo 3 h	Fold Change In Vivo 6 h	Fold Change Ex Vivo 3 h	Fold Change Ex Vivo 6 h
AA485371	BST-2	4.6			
AA598478	C 7				3.1
AA465697	Nip3		2.7	2.0	3.0
AA403083	presenilin				3.3
AA458868	restin		2.7		
AA156940	TFAR19				2.2
AA293570	TNF RSF6		3.9		
H54628	TNF, 10	8.1	11.9		
AA166695	TNF, 13b		5.5		
R70479	TNFA1P2				2.8
AA457114	TNFα-B94		2.7		2.4
Transcription activation and repression					
H99736	CHD-1		2.4		
T72915	CIS3		2.7	2.3	2.8
T71991	CREG		2.2		
R25377	DEK		2.6		
N23047	Dp-2		3.9		
H81219	ELF1			2.1	2.1
AA255811	ELF4		4.0	2.1	
AA164585	ESR1				2.6
T58873	FOSL2				2.4
AA664219	GCCR				3.5
AA699460	hACF1				2.3
N62269	H-1CSBP		2.7	2.1	
AA448261	HMG-1(Y)			2.4	
AA490864	HSF1BP		2.5		2.1
AA099652	HUPF1	5.9	5.2	4.8	6.7
AA490996	IFNGIP1	3.4	4.5	4.3	3.2
AA443090	IRF-7				2.2
AA291389	ISGF3	2.7	2.8	6.2	5.1
W69096	ITF-2				2.0
AA454711	JUNB				2.5
N66278	JUND		2.2		3.2
AA434084	KIAA0593	2.0	2.9	2.5	3.9
AA443722	LBP-1B	2.1			
AA490980	LOC51580			2.1	3.2
AA625793	MAD			2.2	3.1
H86197	MAD			4.9	7.9
AA043501	MAF2		2.2		2.7
H17528	MOP3		2.3		
AA418900	MSL3L1	2.1		2.9	3.1
AA114957	MXI1				2.3
R52824	MYCN				2.8
AA406285	NC2-α		3.2	2.8	2.9
AA279601	NMI		2.9		2.5
AA629687	NRF2		2.1		2.9
AA194811	P2R		2.2		2.2
N63644	PRO0593				2.8
W72621	RYBP				2.4
R41965	RZF 13		3.3	2.1	2.5
AA416971	SNF2H				2.4
AA447481	SP100-HMG	2.4	2.3	2.6	2.3
AA486367	STAT 1	3.1	3.8	5.0	4.5
AA927490	STAT 2		2.1	2.9	4.6
AA863403	STAT 3		3.8	3.0	3.9
AI675516	TFIID		2.4		
H51705	TGIFB			2.1	2.4
AA707195	TRIM 14	2.6	2.0		2.3
AA083407	TRIM 22	3.2	3.6	4.2	3.9
AA490605	ZFHX1B		2.5		
AA150500	HEM45	3.4		7.2	7.7
T71181	ICIL-IRA	9.4	9.7	12.3	6.0
AA776946	ZNF-HRX	2.3	2.6		
RNA processing					
AA600189	ADAR		5.5		
AA464198	E1BAP5			2.2	
H68988	FBL 5		2.0		
R32291	FBL11				2.3
AA490224	FBRNP				3.2
W72293	FLJ10907				2.7
AA455111	HNRNP				2.5
AA490991	HNRNP F		2.1		
AA496741	HNRNP U		2.0		2.8
AA489892	HNRPE1				2.3
AA480866	IS1-RNPL				2.0
AA490490	KIAA0625		2.7	2.2	
AA984940	RNS2		2.3		
AA699361	SAP49		2.3		2.3
W87713	SRp46				2.5
H29484	SSB	2.5	3.8	2.1	3.0
Translation initiation and elongation					
AA577339	4EBP 1				2.2
W60701	EEFIA1		2.1	2.1	3.0
T89301	EEF1A1α				3.9
AA102723	MRP-L15		2.2		
AA010079	PKR	3.5			
AA459727	RPL10				2.3
AA402920	RPL18A				2.7
AA229937	RPL21			2.1	2.4
AA486746	RPL28				2.0
AA687094	RPP0			2.2	2.4
A1732304	RPP1				2.2
AI821339	RPS20				2.4
AA626146	RPS24				2.3
AA625632	RPS27a				2.3
AA156169	RPS3				2.2
AI659736	RPS30				2.1
N91584	RPS6				2.0
AA452556	TRAMP				2.5
A1669217	WSCR 1				2.2
Posttranslational modification					
W46433	ACTR		2.8	2.1	2.7
AA043551	B3GNT5				3.8
AA459123	BAM32	2.2	2.8	2.0	
AA864554	calgranulin B		2.2		
AA436226	CK1 γ2				2.7
AA994790	CKIIβ				2.1
N64862	FYB		2.1		
N66144	FYN				2.2
AA961272	GGTASE-1-β	2.6	2.2		2.8
AA464542	HPTPE		2.8		
N23941	p21			3.0	
N63635	pim-1		2.2	3.2	3.4
AA281667	PKIA				2.6
AA010079	PKR	3.5			
AA278137	PPM2C			2.1	2.6
A1015359	PPPM1A			2.1	2.4
H64260	PRKAG2	2.2		3.1	3.4
AA258396	DT1P1B11			2.2	
AA227142	LITAF				2.5
AA778448	PRKX				2.4
R85257	PTK2 2β	2.2	2.6	2.1	3.2
N25425	RAF				2.3
H24956	RET				2.5
AA426323	RIP			2.3	
AA205665	SET				2.4
AI375353	SGK-1				2.7
T65211	SRPK2				2.4
AA461449	STK3				2.0
T55399	YES1	3.0	4.8	4.1	4.9
H94670	YWHAZ				2.8
Protein folding					
AA126265	calnexin				2.1
AA625981	FKBP-12				2.4
W86653	FKBP51		2.5	2.1	
AA598758	gp96				2.1
AA496105	HSP 40, B6		2.2		2.3
AA620511	HSP 8, 70kD		2.7	2.1	
Protein cleavage and degradation					
AA490894	A-LAP				2.4
AA101760	BLMH				2.5
AA102454	calpain, 2		2.0		2.5
AA676484	calpain, 1				2.1
H80711	caspase 10			2.3	
T95052	caspase, 1		2.5		
AA878951	CD156b				2.5
AA644088	CTSC		2.3		
W73874	CTSL			2.7	2.0
AA156079	CTSS			2.0	3.9
AA599177	cystatin C		2.1		3.3
N52205	FBL3A				2.6
AA406019	IFN-UCRP		8.2	14.8	9.0
AA229587	kallikrein 3				2.3
H73079	KIAA0094				2.2
AA424807	KIAA0107				2.2
R69306	LOC51056	3.4	5.8	3.9	3.7
AA290867	MSP				2.5
R93829	NAP1			2.1	
AA449155	NCUBE1		2.1		2.7
H65394	PA28β		2.6		
N54794	PAI-1			2.5	2.1
AA278759	PRG1		2.9	2.4	2.8
AA284528	PRSS2		2.1		2.4
R71913	PSMα2		2.1		
AA206497	PSMα4		2.7		
AA181300	PSMβ8	2.6	3.7	3.1	2.9
AA430512	SERPINB9		2.2	2.2	3.7
AA664004	TPP1		2.1		
AA292031	UBCH8	4.0	5.9	6.4	7.6
N62725	ubiquilin 2		2.1		
R07443	UNP				2.1
AA425447	YME1-like 1		2.2		3.2
Secretion and trafficking					
AA451895	annexin A5		2.5	2.1	
T71316	ARF4				2.8
N35301	ARF7			2.2	4.1

Table 2. *Continued*

GenBank Accession Number	Gene Name/ Symbol	Fold Change In Vivo 3 h	Fold Change In Vivo 6 h	Fold Change Ex Vivo 3 h	Fold Change Ex Vivo 6 h
AA424743	ZNF 36		2.2		
H99766	ZNF-24		2.9	2.8	3.1
R38383	ZNF36		3.4		4.0
AA994790	CSNK2B				2.1
AA430506	CTSA		2.3		2.5
R92034	KPNA6		2.0		2.0
AA112427	LBC		2.2		
AA456886	MX1	10.2	15.6	12.9	12.7
AA286908	MX2	6.5	9.2	5.9	10.4
AA481531	NUP62	2.3			
N74637	P/CAF			2.0	
AA479100	p76		2.3		
AA281652	PI3K		2.0		
N52178	PP17		2.2		
AA583574	PSOR1				2.1
AA150417	RAB14	2.5	3.3		
R59359	RAB1B				2.6
AA449333	RAB22B		2.4		
R24795	RAB5A			2.0	3.5
R23212	RAB-8b	2.4	2.8		2.3
R76313	RhoG		2.0		2.0
AA425754	SNAP-α			3.0	4.2
AA169814	SNX2		2.5		
AA426027	SNX3				2.4
W15351	SNX6	2.2	2.2	2.6	3.9
R69163	SNX9			2.1	2.1
AI289185	STAP-1			2.5	
R33851	syntaxin 11	2.5	3.6		3.6
T61122	syntaxin 7		2.3		
AA683073	SYT1			2.0	
Metabolism					
AA521292	ABC-1				2.1
AA486080	ATPase				2.1
R78490	CLP				2.7
AA130016	clusterin				2.7
A1016999	COMT				2.0
AA464147	CYSRS				2.0
AA159620	EVDB				2.0
H02307	FLAP				2.1
H67274	G2an				2.2
AA961272	GGTI	2.6	2.2		2.8
R89349	GLS	2.7	3.1	2.9	
A1000103	GLUL		4.4	3.5	4.3
AA406242	GMPR	3.0	3.8	2.7	
AA485362	GSHPX-1				2.4
H67342	GST5				3.3
AA485271	HK1				2.1
A1005515	HK2				3.3
N64010	IPFK-2			2.0	4.3
A1361530	LACS 2	3.0		2.1	3.8
R28548	LACS4		2.5		
AA630104	lipase A			3.0	3.4
A1361330	NMDMC		3.0		2.3
AA404619	NT5B				2.2
AA448157	P450		2.1		2.7
AA010079	PKR	3.5			
HI1054	PKRCD		2.2		
AA778448	PKRX				2.4
AA430382	PNP				2.4
AA608572	PP		2.1		2.2
N25969	PRKAR1A				2.5
H11054	PRKCD		2.2		
A1000971	PRKWNK1				2.1
AA426323	RIP			2.3	
AI375353	SGK1				2.7
AA010811	SSAT		4.8		
H61242	UCP 2				2.0
Biosynthesis					
R72243	2′-5′-OAS	9.8	15.5	16.8	16.0
N34974	ACTIN-2		3.0	2.2	2.7
R66604	actinin, α 4		2.0		2.3
N70038	asr2				2.1
AA455126	ATPS				2.3
T91637	CEPT1			2.0	3.5
H68308	DCTD				2.6
H67274	G2an				2.2
AA486849	GBP1	4.7	7.0	5.9	
AA443688	GCH1		3.7	3.7	
AI000103	GLUL		4.4	3.5	4.3
AA678022	granulin		3.6		3.5
AA487608	HELO1			2.4	2.4
AA485271	HK1				2.1
AI361530	LACS 2	3.0		2.1	3.8
AA630104	lipase A			3.0	3.4
H69561	MAN2A1				2.4
AA858059	MGAT1		3.3	2.3	4.6
AI361330	NMDMC		3.0		2.3
AA961272	PGGT1B	2.6	2.2		2.8
AA608572	PP		2.1		2.2
AA598887	SMC1L1		2.1		
N64431	tubulin, β1	2.1	2.1		
H61242	UCP 2				2.0
Reproduction					
R23524	HSS				2.4
AA431428	inhibin, βB		2.2		
AA280136	JAB			2.6	
Respiration					
AI002403	COX7AL1				2.1
AA463492	CYBB		2.9		3.3
AA027832	HG, α2		3.2		
AA027100	HG, β		2.1		
N68719	HGγ, G	2.8	6.3	3.6	
AA176581	myoglobin			2.1	3.8
Temperature adaptation					
H93550	B3GNT1			2.2	2.0
AA496105	DNAJB6		2.2		2.3
AA598758	gp96				2.1
AA490864	HSF1BP		2.5		2.1
AA620511	HSPA8		2.7	2.1	
Wound healing					
AA490894	A-LAP				2.4
AA451895	annexin A5		2.5	2.1	
AA678022	granulin		3.6		3.5
AA227142	LITAF				2.5
N54794	SERPINE1			2.5	2.1
AA278137	B56G			2.1	2.6
AA459123	BAM32	2.2	2.8	2.0	
AA706829	C20ORF24		2.0		
R37145	RAD51		2.0		2.3
AA487235	VIT-1				2.1
Adenosine triphosphate synthase					
AA486080	ATPase				2.1
AA455126	ATPS				2.3
AA431433	ATPS, e			2.3	
AA251527	KPNB1	2.4	2.7	2.4	3.2
AA449336	PRC1				2.2
R76459	septin 6				2.2
Other signal transduction					
AA017417	1R20	4.1		3.7	
R45977	BCON3				2.2
AA485371	BST-2	4.6			
AA858390	CAPS				2.1
T47814	CD2BP1L				2.7
AA626724	CREM			2.0	
N74236	DXS552E		2.1	2.2	3.4
AA425249	FPR1		3.0		
H00298	GNGT β4		3.6	2.9	
AA460286	GNGT10		2.3		
H72086	GNGT5		2.5		2.2
T67069	GTPBP2		2.1		
AA419251	1FITM1	4.1	6.4	7.2	6.3
AA608576	MAPRE2				2.1
H49511	neurogranin		2.6		
W15297	RAL	2.3	3.0	2.1	2.3
AA279680	RAP1A				2.6
W53015	RAP1B				2.6
T84264	RAP2B				3.0
H48472	SPEC2				2.0
AI289185	STAP-1			2.5	
T71990	WBP-2		2.1		2.1
Other enzymes					
AA490249	atrophin 1	3.3	2.8		
AA682637	C6ST				2.3
AA703453	CHSY1		2.8	2.3	2.2
AA464147	CYSRS				2.0
H68308	DCTD				2.6
AA453293	DPDE4				2.4
AA488340	FACT				2.2
AA779727	FKSG34			2.5	
AA961272	GGT1	2.6	2.2		2.8
AA283864	GLRX	2.2	2.4	2.1	3.0
AI000103	GLUL		4.4	3.5	4.3
AA406242	GMPR	3.0	3.8	2.7	
AA443688	GTPCH1		3.7	3.7	
N32056	heparanase			2.4	2.8
AA664040	IF153	3.1	8.6	7.3	9.4
AA630346	KIAA0212				2.2
AA630104	lipase A			3.0	3.4
AA521303	MAT-II		2.4		2.3
AA455254	MKP-3	2.2		2.1	
N25945	MMTRA1B		4.4		
AI361330	MTHFD		3.0		2.3
AA158236	NADK				2.7

Table 2. *Continued*

GenBank Accession Number	Gene Name/ Symbol	Fold Change			
		In Vivo		*Ex Vivo*	
		3 h	6 h	3 h	6 h
R36586	PP15PIV			2.4	
AA151486	PRS II		2.8	2.8	2.7
N25425	RAF				2.3
H18434	PABP 4				2.2
AA281652	PI3K		2.0		
AA490010	PMCA4			2.2	2.7
AA430382	PNP				2.4
R25612	PO4HB				2.0
AA486289	POLA2				2.5
AA608572	PP		2.1		2.2
AA151486	PRS-II		2.8	2.8	2.7
H86813	TOP1		3.6		
H61242	UCP 2				2.0
Other structural protein					
AA459743	ADD3				2.4
AA488676	CAP-23				3.5
AA449037	CAPZAI		2.3		2.2
AA024656	catenin δ1		3.2		
AA598561	CD164	2.4	2.1	2.7	3.2
AA912458	CHP1		3.9		2.2
R75635	COL5A1		2.3		
R06417	DP III		3.1		2.5
R62817	EPB7			2.6	
AA857944	GHAP		3.2		
R25989	grancalcin		2.8		
AA418994	integrin, α3	2.9	3.0	3.7	4.2
AA186895	KIAA1096				2.1
R06749	KLF3				2.4
AA983462	lamin B1			2.5	
R22977	moesin			2.0	3.0
H73276	p21-ARC				2.7
AA147847	vimentin				2.8
AA459654	WIP				2.9
Unclassified					
T47970	ARPP16				2.2
W47576	ASAHL				3.2
AI268056	Bif-1	2.1	2.9	2.3	3.8
N73536	BM-002				2.3
AA165402	BM-019				2.0
A1286247	BRESI1	8.6	8.6	7.7	8.5
AA670434	BRI3				2.0

GenBank Accession Number	Gene Name/ Symbol	Fold Change			
		In Vivo		*Ex Vivo*	
		3 h	6 h	3 h	6 h
Recombination and repair					
AA478959	ADPRTL1	2.1	2.3	2.4	2.2
AA478273	APEX				3.3
AA489700	CD37				2.7
T98503	CED12		2.4		
T74688	CGI-44	2.4	2.7		2.9
R67222	dl55C23.6	2.5	2.4		
AA431715	EG-1				2.6
AA412509	EHD4			3.9	
HI1658	EphA5				2.0
AA131162	FER1L3		5.2	2.5	
N62244	FLJ30482				2.3
AA213816	FLJ31353		2.9		
N21170	FLN29	4.0		5.1	6.3
AA434102	galectin 9	3.4	5.5	4.1	2.4
T60160	GEC1				2.5
AA872095	GW128				2.2
R10033	H2AFP				2.9
AA486003	H2AFY				3.5
AA683085	HMGB1				2.2
AA598791	HP1-BP74				2.4
H51065	HSOBRGRP		2.2		2.3
AA521232	HSPC022				2.9
H78859	HSPC154				2.1
AA398233	HUMORF13				3.0
N93440	ICB-1		3.1	2.1	
AA489640	IFIT-1		13.7	18.2	11.2
T98558	KDELR2	3.5	3.6	4.6	4.6
N74677	KE2		3.4	2.1	
N39306	kelch-related				2.4
H38995	KIAA0471	2.3	2.2	2.2	2.5
AA495802	KIAA0784				2.2
AA464142	KIAA1554		5.1	3.1	
AA406585	LAPTM5				2.3
AA665912	LCKBP1				2.5
AA279025	lipin 2		3.9	3.5	
AI002103	LOC55871	3.6	2.4		
AA150443	LTBP-4			2.1	
AA479499	LZ16		3.5	2.4	2.8
AA190997	MDS001		2.1		
AA010492	MECP-2				2.1
N32199	melan-A				3.2
AI688349	mitofusin 2				2.1

GenBank Accession Number	Gene Name/ Symbol	Fold Change			
		In Vivo		*Ex Vivo*	
		3 h	6 h	3 h	6 h
H72538	NADPH		2.2		
AA404619	NT5B				2.2
AA187938	P5N1	4.7	6.5	6.3	5.7
AI688014	MLN51				2.7
AA598526	MOP1				2.2
AA410574	MTA1		2.4		
T67103	MY047				3.7
AA064973	myozenin 2	2.5			2.3
H61282	nischarin				2.4
AA452138	NLRR3				2.1
AA115266	NUB1		3.5	4.9	
AA653134	P47ING3				2.2
AA683321	PAR5		2.4		
H72755	PELI1		3.1		
AA047567	PMBP				2.1
AI478107	PORIMIN		3.7	2.5	5.3
AA489386	QK1		2.2		
AA126482	RAD50			2.5	
R38966	RBAF600				2.1
AA465386	RH-II/GuA			2.1	2.5
AA431716	RhoGAP		2.4		
N45131	RO52		2.5		
AA421603	SAMHDI	2.3	3.5	4.8	4.0
W72669	SATB1			2.1	2.7
AA437212	SIGMAIB		3.1		2.3
N94357	slingshot 2				2.2
AA999947	SRL300				2.5
AA025807	SWIP1	2.4	3.9		2.1
AA456316	TACC1				2.6
AA994757	tctex-1	3.5			
R60846	TFG				2.2
AA044633	THIF				3.1
AA455270	TIF2		3.0		3.9
H96734	TIN2			2.0	
AA428607	TSPY-like				2.0
AA173189	TXNDC1		4.4	3.4	4.9
AA872011	URAX1	2.1			
AA428341	verprolin			2.0	3.9
N62339	VMP1		2.8		2.1
N49763	WTAP				2.9
H68938	Xm1/Dhm2		3.4	2.0	4.3

NOTE. For different clones derived from the same gene, only one clone was listed.

mediates homing of lymphocytes to lymph nodes where these cells are activated, was consistently up-regulated under all conditions examined. Other genes up-regulated in PBMCs include perforin and granzyme B, two proteins involved in cytotoxic T-cell or natural killer cell–mediated cytotoxicity,[21] an important effector function of the immune responses against viral infection. Furthermore, multiple genes for proinflammatory cytokines and their receptors were up-regulated, suggesting that IFN-α enhances the inflammatory response. In addition to genes directly relating to immune cell functions, we found up-regulation of 56 genes relating to differentiation and proliferation, as well as large numbers of genes relating to different signal transduction pathways, suggesting that IFN-α may regulate growth of immune cells. Of note, it has been reported that type I IFN accelerated turnover of memory T cells[22] and protected activated T cells from apoptosis.[23,24] In summary, our findings revealed a profound effect of IFN on human immune cells. Cellular immunity, including innate and adaptive cell-mediated

Table 3. Number of IFN-α Regulated Genes in Different Functional Categories

Function/Pathway	Number
Immunity	88
Antigen recognition	5
Antigen processing and presentation	14
Cell-mediated cytotoxicity	7
Inflammatory response	22
Other immunity and defense	40
Cell growth and death	92
Differentiation and proliferation	56
Cell cycle	13
Apoptosis	23
RNA synthesis and processing	68
Transcription activation and repression	52
RNA processing	16
Protein synthesis and processing	89
Translation initiation and elongation	19
Posttranslational modification	31
Protein folding	6
Protein cleavage and degradation	33
Metabolism and biosynthesis	60
Metabolism	36
Biosynthesis	24
Other functions	138
Unclassified	85

immune responses, is believed to play a critical role in the clearance of HCV.[25-28] Our data suggest that IFN-α treatment enhances cell-mediated immune response against HCV by up-regulating expression of multiple genes. The current analysis does not identify the cellular source for the response genes, which could include B cells, T cells, natural killer cells, and monocytes. Future microarray analyses of fractionated PBMCs should provide information on the effect of IFN on each of these subsets.

Several previously identified ISG in different functional pathways have been shown to suppress virus growth at various stages of the viral replication cycle. These include IFN-inducible double-strand RNA-dependent protein kinase,[29,30] IFN-inducible 2′, 5′-oligoadenylate synthetase,[31,32] IFN-inducible Mx proteins,[33,34] and RNA-specific adenosine deaminase.[35,36] Of note, the antiviral effect of these IFN-inducible proteins only has been shown for a narrow range of viruses. In this study, RNA-dependent protein kinase, IFN-inducible 2′, 5′-oligoadenylate synthetase, Mx proteins, and RNA-specific adenosine deaminase were all significantly up-regulated in PBMCs after IFN-α administration. Because an *in vitro* culture system is not available for HCV, the effect of IFN-α on the replication of HCV has not been studied directly *in vitro*. However, the replication of a subgenomic replicon of HCV in a human hepatoma cell line was suppressed efficiently by IFN-α,[37] which supports the notion that IFN-α can suppress HCV replication directly, although the cellular gene(s) involved in this suppression are unknown. A recent microarray study on primary human hepatocytes identified 43 genes that were up-regulated at least 2-fold by IFN-α.[38] Of these, 19 were up-regulated in the PBMCs in our study, including all the 5 putative antiviral ISG in the primary hepatocytes. These results suggest that at least some of the hepatocyte IFN response responsible for the direct anti-HCV effect of IFN may be mimicked by the IFN response in PBMCs. The large number of ISG identified in this study should serve as promising candidates for further analysis.

As shown in Table 3, the up-regulated genes involve the critical biologic functions of cell proliferation, differentiation, apoptosis, cellular metabolism of protein, nucleic acid, and carbohydrate molecules, as well as multiple signal transduction networks. This reflects the enormous breadth and diversity of the cellular pathways affected by systematically administrated IFN-α. IFN-α is produced during virus infection by infected cells as well as certain immune cells.[39,40] Although little information is available, it is likely that the natural expression of IFN-α is at a low level, transient, and may be limited to a small number of cells. Cytokines are thought to act locally around their producers,[41] suggesting that the effect of the naturally produced IFN-α may be far more limited in terms of affected cells or organs. On the other hand, when IFN-α is given systematically as a therapeutic agent, its profound effects on gene expression, as shown herein, can act generally throughout the body. Any consequence of IFN-α treatment, including its success or failure in clearing the virus as well as its adverse side effects, will not be fully understood without being examined in the context of system-wide global gene expression.

Searching for Differentially Regulated IFN-α–Inducible Genes Between Responders and Nonresponders. The long-term goal of this study was to identify IFN-α–regulated genes associated with different treatment outcome. Six of the 7 patients enrolled in this initial study have finished treatment and have been followed-up for 6 months after treatment. There are 2 end-of-treatment responders, one sustained and the other relapsed (Table 1). We have performed a 2-class SAM analysis based on available microarray datasets (Table 1) to compare the responders P1 and P2 versus nonresponders P3, P4, P5, and P6, using 3-hour *in vivo* datasets (one microarray for each patient). No genes were identified as differentially regulated by IFN-α between the 2 groups at FDR less than 50%. The negative result is not a surprise considering the small sample size ($n = 2$ for the responder group), which was barely enough for any statistical analysis, as well as the heterogeneity of the responder group, which actually comprised a sustained responder and a relapser. A larger sample size will increase

the sensitivity of detecting genes differentially regulated by IFN-α between the patients with different treatment outcomes.

Conclusion

We used cDNA microarrays to profile gene expression in human PBMCs treated *in vivo* and *ex vivo* with IFN-α and the data were analyzed statistically to identify genes significantly regulated by IFN-α. The PBMC population we studied contained peripheral lymphocytes and monocytes and hence represents a component of the immune system that is believed to play a critical role in the control of viral infection. These cells were isolated directly from chronic hepatitis C patients before and after IFN-α injection (*in vivo* study), or incubated with IFN-α (*ex vivo* study). Because PBMC is a heterogeneous population consisting of various subsets of cells that may respond to IFN-α differently, the information generated in PBMCs is likely to be a useful reference for IFN-α–regulated gene expression in other primary human cells or organs.

We have identified a large number of genes with statistically significant changes in their expression in response to IFN-α. It needs to be stressed that although many changes are relatively small and could be missed easily by conventional techniques, the synergetic effect of several key genes may generate a profound impact on the outcome of IFN-α treatment for HCV infection. Previous studies have suggested multiple viral and host factors affecting the outcome of IFN therapy, including HCV genotype, viral load, race, age, and so forth.[42] It is possible that much of the contribution of these factors will be reflected in the IFN response of host cells. As the first step in our efforts to identify IFN-regulated genes in association with treatment outcome, this study has focused on the feasibility of the microarray strategy but has not included enough subjects to address the difference in response patterns between patients and how these differences correlate with treatment outcome. However, based on the findings in this study, we predict that compared with patients who fail to respond to treatment, patients who respond favorably will have a more vigorous (greater number and/or fold increase) induction in the expression of genes related to direct suppression of HCV as well as in genes related to immune functions. We further predict that such signature genes, or genes associated with different treatment outcome, can be identified by using a 2-class SAM analysis. Once identified, these signature genes might be used as a predictor for the outcome of therapy. The work described in this report sets a foundation for addressing this important issue.

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