

Computational networks of activating transcription factor 3 gene in Huh7 cell lines and hepatitis C virus-infected Huh7 cell lines

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Abstract. Activating transcription factor 3 (ATF3) is an adaptive-response gene of the ATF family. ATF3 activity may be induced in response to a number of different stress-associated signals and ATF3 is involved in a variety of cellular processes. However, the functions of ATF3 and its molecular networks in human hepatoma cells lines and hepatitis C virus-infected Huh7 (HCV-Huh7) cells are not well understood. In the present study, ATF3 regulatory networks in Huh7 and HCV-Huh7 cell lines were established using the linear programming-based GRNinfer software and molecule annotation system 3.0 software. The gene expression omnibus dataset, GSE20948, was analyzed. The resulting network consisted of clusters located upstream and downstream of ATF3 in Huh7 and HCV-Huh7 cell lines. Using the annotation, visualization and integrated discovery (DAVID) software, 10 activation and 2 inhibition enriched functional annotation clusters were identified downstream of ATF3 in HCV-Huh7 cells. However, there were no enriched functional annotation clusters identified upstream of ATF3 in HCV-Huh7 cells. Furthermore, no clusters were identified downstream nor upstream of ATF3 in Huh7 cells. Gene ontology term and Kyoto encyclopedia of genes and genomes pathway analyses demonstrated that ATF3 may be involved in a number of biological processes, in particular, in metabolism regulation in HCV-Huh7 cells. It is hypothesized that the ATF3 pathway may be activated in Huh7 cells following HCV infection and that it is a potential 'hub' in the network of HCV-Huh7 cells.

Introduction

Hepatitis C virus (HCV) infection affects 170 million people worldwide and as a major cause of liver disease worldwide, HCV is a potential cause of substantial morbidity and mortality in the future and therefore, a health serious health concern (1). Approximately 80% of patients with HCV fail to clear the virus and <10% may develop severe liver diseases, such as chronic hepatitis, liver cirrhosis and hepatocarcinoma, which reduce the chance of patient survival (2). Understanding HCV pathology and the development of novel drugs with which to treat patients with HCV is an ongoing challenge.

Microarrays and different types of high-throughput approaches, for example, high-throughput RNA-Seq and high-throughput DNA-Seq, for gene expression analysis have led to an improved understanding of the processes underlying HCV infection. These approaches are advantageous compared with traditional approaches, which are restricted to analyzing small numbers of genes (3). Microarray technology does not require gene selection in advance, meaning that the method is less biased and is capable of identifying genes that are modified when cells are exposed to environmental changes (4). Microarray technology aims to establish gene regulatory networks and to identify interactions among genes and their products. Carefully analyzed networks are used to identify correlated genes that are associated with the same biological processes or pathways, and to infer interactions among molecules, such as physical association, metabolite flow, regulatory associations and co-expression (5). In the present study, GRNinfer software was used in order to establish a gene regulatory network and to identify molecular interactions, including activation and inhibition, in the activating transcription factor 3 (ATF3) signaling pathway in healthy Huh7 (Huh7) and HCV-infected (HCV-Huh7) cell lines.

Materials and methods

Microarray. The gene expression profile data GSE20948 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE20948>) were extracted from the public Gene Expression Omnibus database (<http://www.ncbi.nlm.nih.gov/gds/>). Microarray analysis was performed for 14 Huh7

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samples (2 samples, 6 h; 3 samples, 12 h; 3 samples, 18 h; 3 samples, 24 h; 3 samples, 48 h.) and 14 HCV-Huh7 samples (2 samples, 6 h; 3 samples, 12 h; 3 samples, 18 h; 3 samples, 24 h; 3 samples, 48 h); the latter had been infected with HCV for 6, 12, 18, 24 or 48 h. The platform of the data was GPL 570 (HG-u133-plus-2) affymetrix human Genome u 133 2.0 array.

Gene selection algorithms. Fifty HCV-Huh7 molecular markers were identified using Multiexperiment Viewer (<http://www.tm4.org/mev.html>; Version 4.8.1-windows), which belongs to TM4, in order to conduct a significant analysis of microarrays (SAM). The TM4 suite consists of four major applications, including Microarray Data Manager, TIGR_Spotfinder, Microarray Data Analysis System. This software is a free, open-source software released under the Artistic license, and is OSI certified. SAM is a statistical technique, used to identify significant genes in a set of microarray experiments. The explanatory variable is gene expression measurement from a set of microarray experiments, and the response variable is a grouping, such as mock-infected or infected. Microarray raw data CEL files were processed using expression console software of Affymetrix, Inc. (Santa Clara, CA, USA). In the present study, 50 significant genes using ATF3 as a target gene, which was of interest, were further analyzed.

Network establishment of candidate genes. ATF3 gene networks were constructed using GRNinfer (6) and GVedit (<http://portableapps.com/node/38245>; version: 2.38) tools. GRNinfer is a novel mathematical method based on linear programming and a decomposition procedure that infers gene networks. The method ensures derivation of the most consistent network structure reducing the issues associated with data scarcity, yet improving reliability. The following equation represents all of the possible networks for the same dataset: $J=(X^T A)U\Lambda^T V^T + YV = J + YV^T$. Where $J=(J_{ij})_{n \times m} = \partial f(x)/\partial x$ is an $n \times m$ Jacobian matrix or connectivity matrix, $X=(x(t_1), \dots, x(t_m))$ and all $n \times m$ matrices with $x'_i(t_j)=[x_i(t_j+I)-x_i(t_j)]/[t_j+I-t_j]$ for $i=1, \dots, n; j=1, \dots, m$. $X(t)=(x_1(t), \dots, x_n(t))^T \sum R^n$, $a=(a_1, \dots, a_n)^T \sum R^n$, $x_i(t)$ is the expression level (mRNA concentrations) of gene i at time instance t , $y=(y_{ij})$ is an $n \times n$ matrix, where y_{ij} is zero if $e_j=0$. U is a unitary $m \times n$ matrix of left eigenvectors, $\Lambda = \text{diag}(e_1, \dots, e_n)$ is a diagonal $n \times n$ matrix containing the n eigenvalues and V^T is the transpose of a unitary $n \times n$ matrix of right eigenvectors.

The parameters were λ , 0.0 and threshold, 1×10^{-9} .

Functional annotation clustering. The database for annotation, visualization and integrated discovery (DAVID; <http://www.david.niaid.nih.gov>) was used. The DAVID gene function clustering tool provides representative annotation and gene ontology (GO) term enrichment analysis, which separates genes into different enrichment score groups according to the gene annotation collective frequency occurrence (7,8).

Molecule annotation system 3.0 (MAS 3.0). MAS (<http://bioinfo.capitalbio.com/mas3/>) is an analysis platform, which adds biological function annotation to high throughput microarray data. By integrating the relevant annotation information from a number of public information databases, MAS synthesizes biological data, including genes, proteins, functions, expression, protein interactions, signaling pathways, diseases and methylation.

MAS enables the incorporation of genetic data from Genebank, European molecular biology laboratory, SwissPort, GO, KEGG, BioCarta, gene map annotator and pathway profiler (GenMapp), mirBase, expected progeny differences, HRPD, MIND, BIND, Intact, TRANSAC, UniGene, single nucleotide polymorphism database, OMIM, InterPro, HUGO, mouse genome informatics and rat genome database.

Results

Gene enrichment analyses. ATF3 was one of fifty genes which were significantly differentially expressed between Huh7 and HCV-Huh7 cell lines (fold change=8.782885; Table I). Gene GO term enrichment analysis for ATF3 demonstrated that the molecular function of ATF3 is associated with transcription corepressor activity, protein binding, sequence-specific DNA binding and protein dimerization activity. ATF3 biological processes are associated with DNA-dependent transcription regulation. ATF3 cellular component is localized in the nucleus and nucleolus. GenMAPP analysis demonstrated that ATF3 is associated with smooth contraction, hypertrophy model, NetPath 5 and Hs transforming growth factor $\beta 1$ (TGF- β) NetPath 7, and that it exhibits transcription cofactor activity. Disease analysis demonstrated that ATF3 is associated with shock, leukemia, colorectal cancer, neoplasm metastasis, nervous system disease, stomach cancer, necrosis, leukemia T cell, lymphoma non-hodgkins, malignant neoplasm of the breast, hyperalgesia, episodic ataxia type 2 and hereditary ataxia overview.

Identification of genes upstream and downstream of ATF3.

Using GRNinfer, a cell network was constructed, which included gene clusters upstream and downstream of ATF3 in Huh7 and HCV-Huh7 cell lines. According to DAVID software analysis, 12 gene clusters were identified downstream of ATF3 in HCV-Huh7 cells (10 activation and 2 inhibition; Table II). Using an MAS 3.0 software GO analysis, ATF3 annotation regulation networks were enriched. The upstream pathway of ATF3 in Huh7 cells included the activation of stanniocalcin 2 cationic amino acid transporter, Y+ system member 1 (SLC7A1) and insulin receptor substrate 2 (IRS2), involving 19 GO terms and three KEGG pathways, and inhibition of four and a half LIM domains 2 (FHL2), involving five GO terms. The downstream pathway of ATF3 in Huh7 cells included inhibition of zinc finger protein 295 (ZNF295), involving two GO terms. The upstream pathway of ATF3 in HCV-Huh7 cells included the activation of inhibin β E chain (INHBE), asparagine synthetase (ASNS) and SLC7A1, involving ten GO terms and four KEGG pathways, and the inhibition of reticulocalbin 1, EF-hand calcium binding domain (RCN1), involving one GO term. The downstream pathway of ATF3 in HCV-Huh7 cells included the activation of the following genes: Brain-derived neurotrophic factor, cold inducible RNA binding protein, FHL2, fatty acid binding protein 3, interferon regulatory factor 9, InaD-like (INADL), IRS2, IRS1, kruppel-like factor 10, LOC100506392, LY6/PLAUR domain containing 1, prion protein, peroxisome proliferator-activated receptor γ coactivator 1- α , RAR-related orphan receptor A, phospholipase A1 member A, SFT2 domain containing 3, SMAD family member 5, TGF β 1 induced transcript 1,

Table I. Fifty genes exhibiting significant differential expression between Huh7 and HCV-Huh7 cell lines identified using SAM.

Gene ID	Gene	Fold change
201010_s_at	TXNIP	21.518297
203438_at	STC2	20.873947
210587_at	Inhibin β E chain	19.26132
201008_s_at	TXNIP	15.231962
203439_s_at	STC2	14.20261
201009_s_at	TXNIP	13.7505045
238029_s_at	SLC 16, member 14	11.429825
205047_s_at	Asparagine synthetase	11.315236
231202_at	Aldehyde dehydrogenase family 1 member	11.254294
225283_at	Arrestin domain-containing protein 4	10.549104
228653_at	SAMD5	9.061874
202672_s_at	Activating transcription factor 3	8.782885
201300_s_at	Prion protein	7.5039783
212909_at	LY6/PLAUR domain containin 1	7.415823
218332_at	Brain expressed, X-linked 1	7.3808937
203372_s_at	SOCS2	7.347132
219195_at	Peroxisome proliferator-activated receptor γ coactivator 1- α	6.9846096
212810_s_at	SLC 1 member 4	6.877748
210426_x_at	RORA	6.6006436
209183_s_at	Chromosome 10 open reading frame 10	6.530727
226682_at	RORA	6.4361844
202393_s_at	Kruppel-like factor 10	6.4241524
214285_at	Fatty acid binding protein 3	6.3771186
203882_at	Interferon regulatory factor 9	6.129926
221523_s_at	Ras-related glutamic-pyruvate transaminase binding D	6.011061
212295_s_at	SLC 7 (cationic amino acid transporter, Y+ system) member 1	5.976633
1554008_at	Oncostatin M receptor	5.8743997
218851_s_at	SFT2 domain containing 3, WD repeat domain 33	5.831587
223681_s_at	InaD-like	5.778195
203373_at	SOCS2	5.741121
209651_at	Transforming growth factor β 1 induced transcript 1	5.6655536
201063_at	Reticulocalbin 1, EF-hand calcium binding domain	5.624495
1569433_at	SAMD5	5.4978223
206382_s_at	Brain-derived neurotrophic factor	5.459223
221530_s_at	Basic helix-loop-helix family, member e41	5.424661
228708_at	Ras-related protein Rab-27B	5.3274593
209185_s_at	IRS 2	5.273203
209610_s_at	SLC 1 member 4	5.230212
214755_at	UDP-N-acetylglucosamine pyrophosphorylase 1-like 1	5.206161
202847_at	Phosphoenolpyruvate carboxykinase 2	5.199079
227037_at	Phospholipase D family, member 6	5.078215
225539_at	Zinc finger protein 295	5.0584846
210479_s_at	RORA	5.0370793
212290_at	SLC 7 (cationic amino acid transporter, Y+ system) member 1	5.0187483
228519_x_at	Cold inducible RNA binding protein	4.95052
219584_at	Phospholipase A1 member A	4.935018
242979_at	IRS 1	4.919549
202949_s_at	Four and a half LIM domains 2	4.856401
1568813_at	LOC100506392	4.792747
212811_x_at	SLC 1 member 4	4.7892227

TXNIP, thioredoxin interacting protein; STC2, stanniocalcin 2; SAMD5, sterile α motif domain containing 5; SOCS2, suppressor of cytokine signaling 2; SLC, solute carrier family; RORA, RAR-related orphan receptor A; IRS, insulin receptor substrate; SAM, significant microarrays; HCV, hepatitis virus; Huh7 cell line, hepatoma cell line; HCV-Huh7 cell line, HCV-infected Huh7 cell line.

Table II. Functional annotation clusters (activation and inhibition) downstream of activating transcription factor 3 in hepatitis C virus-infected Huh7 cells.

A, Activation		
Cluster	Annotation	Enrichment
1	Positive regulation of fatty acid oxidation; positive regulation of glucose metabolic process; positive regulation of carbohydrate metabolic process; regulation of carbohydrate biosynthetic process; positive regulation of fatty acid metabolic process; regulation of fatty acid oxidation; positive regulation of macromolecule biosynthetic process; regulation of glucose metabolic process; positive regulation of cellular biosynthetic process; regulation of cellular carbohydrate metabolic process; positive regulation of biosynthetic process; regulation of fatty acid metabolic process; regulation of cellular ketone metabolic process; positive regulation of macromolecule metabolic process; adipocytokine signaling pathway; regulation of lipid metabolic process; Insulin signaling pathway; chemical homeostasis; homeostatic process.	2.93
2	Regulation of cell proliferation; response to insulin stimulus; enzyme linked receptor protein signaling pathway; response to hormone stimulus; response to organic substance; response to endogenous stimulus; response to peptide hormone stimulus; cell surface receptor linked signal transduction.	1.98
3	Regulation of cell proliferation; negative regulation of cell proliferation; positive regulation of cell differentiation; positive regulation of developmental process.	1.94
4	Androgen receptor binding; positive regulation of macromolecule biosynthetic process; androgen receptor signaling pathway; steroid hormone receptor binding; positive regulation of cellular biosynthetic process; activator; transcription factor; steroid hormone receptor signaling pathway; positive regulation of macromolecule metabolic process; intracellular receptor-mediated signaling pathway; nuclear hormone receptor binding; hormone receptor binding; positive regulation of transcription, DNA-dependent; positive regulation of RNA metabolic process; transcription regulator activity; transcription coactivator activity; positive regulation of transcription; transcription; positive regulation of gene expression; transcription from RNA polymerase II promoter; positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process positive regulation of nitrogen compound metabolic process; nucleus; transcription regulation; transcription, DNA-dependent; RNA biosynthetic process; regulation of RNA metabolic process; intracellular signaling cascade; transcription cofactor activity; transcription activator activity; transcription factor binding; phosphoprotein; transition metal ion binding; zinc-finger; zinc ion binding; DNA binding; cation binding; ion binding.	1.57
5	Intracellular signaling cascade; cell junction; plasma membrane part. 1.07	1.04
6	Chemical homeostasis; homeostatic process; protein complex biogenesis; protein complex assembly; macromolecular complex assembly; macromolecular complex subunit organization.	0.81
7	Activator; nuclear lumen; intracellular organelle lumen; organelle lumen; membrane-enclosed lumen.	0.81
8	Regulation of apoptosis; regulation of programmed cell death. 0.61	
9	Mutagenesis site; alternative splicing; splice variant. 0.35	
10	Plasma membrane; glycosylation site: N-linked; signal; glycoprotein; signal peptide; disulfide bond; disulfide bond; intrinsic to membrane; membrane.	0.27
B, Inhibition		
Cluster	Annotation	Enrichment
1	Response to endogenous stimulus; response to organic substance; regulation of apoptosis; regulation of programmed cell death; regulation of cell death.	1.47
2	Glycosylation site: N-linked; glycoprotein; signal; signal peptide.	0.54

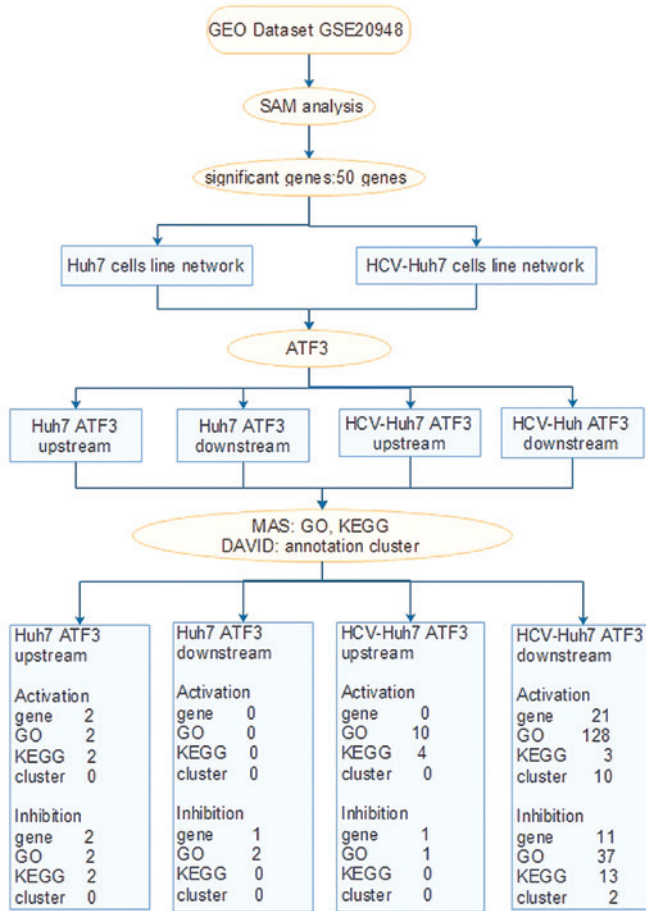


Figure 1 ATF3 network construction and analysis processes. ATF3, activating transcription factor 3; GO, gene ontology; TXNIP, thioredoxin interacting protein; STC2, stanniocalcin 2; SAMD5, sterile α motif domain containing 5; SOCS2, suppressor of cytokine signaling 2; SLC, solute carrier family; RORA, RAR-related orphan receptor A; IRS, insulin receptor substrate; DAVID, database for annotation, visualization, and integrated discovery; KEGG, kyoto encyclopedia of genes and genomes; SAM, significant analysis of microarrays; GEO, gene expression omnibus; HCV, hepatitis C virus; HCV-Huh7 cells, HCV-infected Huh7 cells; Huh7 cells, hepatoma cells.

UDP-N-acetylglucosamine pyrophosphorylase 1-like 1, ZNF295 and SLC7A1, involving 128 GO terms and 13 KEGG pathways. The downstream pathway in HCV-Huh7 cells included the inhibition of the following genes: SLC7A1, arrestin domain-containing protein 4, ASNS, chromosome 10 open reading frame 10, INHBE, phosphoenolpyruvate carboxykinase 2, PCK2, RCN1, stanniocalcin 2, suppressor of cytokine signaling 2, SLC1A4 and thioredoxin interacting protein, involving 37 GO terms and 13 KEGG pathways.

Functional annotation clustering analyses. Ten activation and two inhibition annotation clusters were enriched downstream of ATF3, in HCV-Huh7 cells. The enrichment scores of the activation clusters ranged from 0.27-2.93, and the two inhibition clusters were 0.54 and 1.47. Among the activation clusters, the highest enrichment score (cluster 1; 2.93) was associated with positive metabolism regulation, such as fatty acid oxidation, glucose metabolism, carbohydrate metabolism, cellular carbohydrate metabolism, fatty acid metabolism, macromolecule biosynthesis, cellular

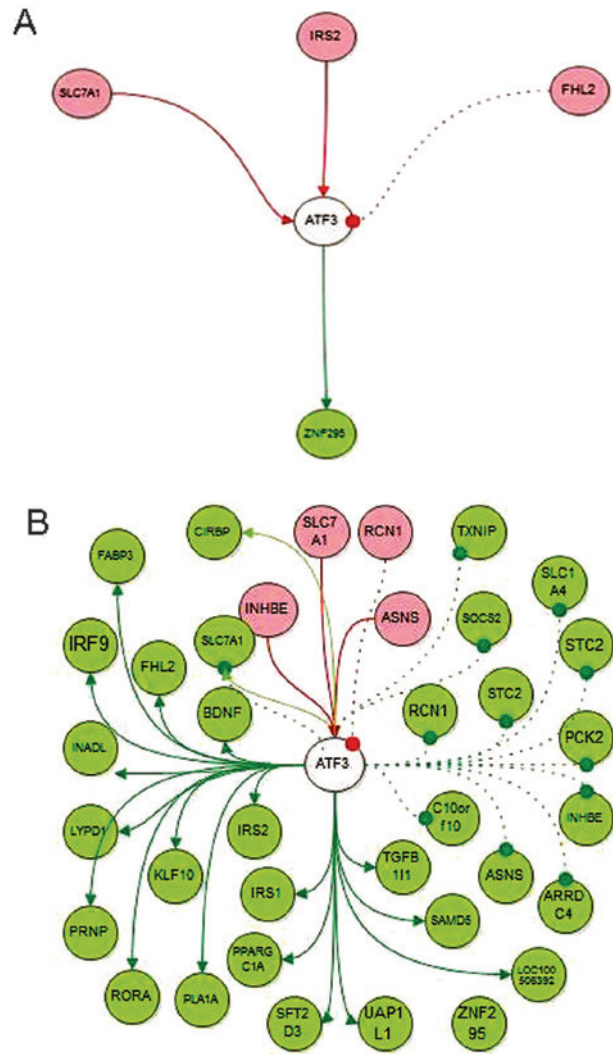


Figure 2. ATF3 network in healthy HUH7 and HCV-infected Huh7 cell lines. (A) ATF3 network in Huh7 cells; (B) ATF3 network in HCV-Huh7 cell lines. Red circle with gene name indicates the genes upstream of ATF3; Green circle with gene name indicates the genes downstream of ATF3; Red solid line with red arrow indicates the activation of ATF3 by the gene upstream to ATF3; Red dashed line with red dot indicates the inactivation of ATF3 by the gene upstream; Green solid line with green arrow indicates the activation role of the gene in the downstream by ATF3; Green dashed line with green dot indicate the inhibition of the gene in the downstream by ATF3. ATF3, activating transcription factor 3; HCV, hepatitis C virus; TXNIP, thioredoxin interacting protein; STC2, stanniocalcin 2; SAMD5, sterile α motif domain containing 5; SOCS2, suppressor of cytokine signaling 2; SLC1A4, solute carrier family member 4; RORA, RAR-related orphan receptor A; IRS, insulin receptor substrate. INHBE, inhibin β E; ASNS, asparagine synthetase; ARRDC4, arrestin domain-containing protein 4; LYPD1, LY6/PLAUR domain containin 1; PPARGC1A, peroxisome proliferator-activated receptor γ coactivator 1- α ; KLF10, kruppel-like factor 10; FABP3, fatty acid binding protein 3; IRF9, interferon regulatory factor 9; SLC7A1, SLC 7 (cationic amino acid transporter, Y+ system) member 1; C10orf10, chromosome 10 open reading frame 10; SFT2D3, SFT2 domain containing 3; INADL, inaD-like; TGTB11, transforming growth factor β 1 induced transcript 1; RCN1, reticulocalbin 1, EF-hand calcium binding domain; BDNF, brain-derived neurotrophic factor; UAP1L1, UDP-N-acetylglucosamine pyrophosphorylase 1-like 1; PCK2, phosphoenolpyruvate carboxykinase 2; ZNF295, zinc finger protein 295; CIRBP, cold inducible RNA binding protein; PLA1A, phospholipase A1 member A; FHL2, four and a half LIM domains 2; PRNP, prion protein.

biosynthesis, lipid metabolism, fatty acid metabolic regulation, macromolecule metabolism and androgen receptor

binding. The activation clusters exhibiting enrichment scores 0.27-1.98 were associated with regulating cell proliferation, transcription, DNA-dependent developmental processes, gene expression, responses to stimuli of insulin, endogenous organic substances, peptide hormones, androgen receptor binding, androgen receptor signaling pathways, steroid hormone receptor binding, nuclear hormone receptor binding, intracellular signaling cascades, zinc-finger transcription factors and metal-binding. Therefore, it is hypothesized that ATF3 is inactive in Huh7 cells and is activated upon HCV infection, thereby regulating a number of cytokine functions, metabolic pathways and signal transduction. The present study identified 4 genes that were upstream of ATF3 and 33 that were downstream of ATF3 in HCV-Huh7 cells. Therefore, ATF3 may be an important regulatory factor involved in the pathological reactions of Huh7 cells undergoing the initial stages of infection with HCV.

Fig. 1 demonstrates the ATF3 network construction and analysis processes.

Discussion

In order to understand the involvement of ATF3 in HCV-infected Huh7 cells, an ATF3 GO network was established for Huh7 and HCV-Huh7 cell lines. HCV infection leads to complex biological responses and, therefore, the subsequent genetic interactions in the infected cells are typically non-linear. However, gene networks may only be predicted based on linear equations, due to the limited understanding of biological processes required for more complex statistical analyses (9-12). In the present study, GRNinfer, a linear programming and decomposition software, was used in order to analyze and compare ATF3 networks in Huh7 and HCV-Huh7 cell lines. Constructed networks included upstream and downstream gene clusters of ATF3 in Huh7 and HCV-Huh7 cell lines (Fig. 2). Furthermore, using DAVID and MAS 3.0 software, the KEGG pathways and GO terms of four gene clusters were enriched. The results of the present study demonstrated that downstream pathways of ATF3 in HCV-Huh7 cells were significantly enhanced.

The results of the present study suggested that ATF3 may be inactive in Huh7 cells and activated following HCV infection of Huh7 cells. These results are in accordance with those observed in previous studies. ATF3 expression levels were found to be low in other cell lines or tissue (13) and were shown to rise when cells were subjected to a number of different stresses, including ischemia and trauma, as well as exposure to toxic chemicals. ATF3 mRNA expression was not detected, or remained at low levels, in a number of cell types (14). The present study demonstrated that 4 genes were upstream or downstream of ATF3 in Huh7 cells, whereas 36 genes were upstream or downstream of ATF3 in HCV-Huh7 cells. Therefore, ATF3 may be inactive in healthy Huh7 cells while it is activated in HCV-infected Huh7 cells (Fig. 2). These observations require further investigation.

Four genes were identified upstream, while 33 genes were downstream of ATF3 in HCV-Huh7 cells. Therefore, ATF3 may be associated with HCV pathology in Huh7 cells. A previous study suggested that ATF3 may represent an immediate early responsive gene, which is predominantly activated at a transcriptional level (15).

Extensive studies have shown that ATF3 is involved in immune regulation (16-23), endocrine regulation (24-27), tumorigenicity (28-32), apoptosis, cell cycling (33-37) and inflammation (17,34,38-43). This suggests that ATF3 is associated with host defensive responses to inflammation, viruses and cancer. These processes require the interactions of certain cellular pathways, which influence disease outcomes. ATF3 is an adaptive-response gene, which is involved in a number of cellular processes, and accommodates cellular changes by transducing signals from various receptors via activating or repression of gene expression. ATF3 may also regulate host defense mechanisms (13). According to DAVID functional annotation clustering analysis, no annotation clusters were identified downstream of ATF3 in Huh7 cells nor were there clusters identified upstream in Huh7 and HCV-Huh7 cells. However, ten activation annotation clusters were enriched downstream of ATF3 in HCV-Huh7 cells that exhibited enrichment scores from 0.27-2.93 and two inhibition annotation clusters were enriched downstream of ATF3 in HCV-Huh7 cells that exhibited enrichment scores of 0.54 and 1.47. In addition, analyses using MAS 3.0 GO and KEGG, suggested that 128 GO terms and 13 KEGG pathways are involved downstream of ATF3 in HCV-Huh7 cells and 37 GO terms and 13 KEGG pathways were inhibited. It is hypothesized that ATF3, as an adaptive-response gene, may be involved in the pathophysiological responses of Huh7 cells to HCV infection.

In addition to liver injury and hepatocellular carcinoma, HCV infection is associated with fatty degeneration of the liver, suggesting that hepatitis C is a metabolic disease (44,45). Considerable evidence suggests that HCV infection is associated with metabolic syndromes, which referred to constellation of problems, including insulin resistance, obesity, hypertension and hyperlipaemia. Fatty degeneration of the liver and insulin resistance may be important in HCV-induced metabolic syndrome and a molecular mechanism underlying this process is a virus-induced metabolic disorder of fat in the liver (46). The liver is important for the control of lipogenesis, gluconeogenesis and cholesterol metabolism. A number of investigations into pathological examination have highlighted the importance of metabolic function in liver diseases. The process of lipid metabolism is important in the proliferation of HCV infection (47).

As a cyclic AMP response element-binding protein family member, ATF3 participates in a range of cellular processes. Recent studies have shown that changes in ATF3 activity is associated with energy balance. Homeostasis imbalance may lead to organ growth failure, retardation and metabolic disorder. A loss of ATF3 in *Drosophila* was found to result in chronic inflammation and primary starvation responses, including lipid overloading in the fat bodies of the larval epithelium, which may lead to energy imbalance and death (48). Kim *et al* (49) showed that ATF3 was involved in a cholesterol-sensing network.

In the present study, DAVID cluster analysis found that the cluster with the highest enrichment score (2.93) was downstream of ATF3 in HCV-Huh7 cells (Table II). This suggests that ATF3 not only activates lipid metabolism, but also positively regulates fatty acid metabolic, macromolecular biosynthetic and cellular biosynthetic processes. The results of the present study showed that ATF3 may influence metabolic processes in HCV-Huh7 cells. The current study provides a

novel perspective for exploring the association of ATF3 in HCV infection of Huh7 cells. However, further research is required in order to explore how ATF3 is associated with metabolic processes and the regulatory functions of ATF3.

In conclusion, an ATF3 network was constructed for Huh7 and HCV-Huh7 cell lines and gene functional annotation and enrichment cluster analyses were performed. The results of the present study suggested that ATF3 is inactive in healthy Huh7 cells and activated following HCV infection. ATF3 may contribute to the initial pathological responses to HCV infection in Huh7 cells. ATF3 may be associated with a number of processes, in particular lipid metabolism, during acute HCV infection of Huh7 cells.

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