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Modulation of gene expression in MHCC97 cells by interferon alpha

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Abstract

AIM: To elucidate the molecular mechanisms of the inhibitory effects of IFN-α on tumor growth and metastasis in MHCC97 xenografts.

METHODS: Three thousand international units per milliliter of IFN-α-treated and -untreated MHCC97 cells were enrolled for gene expression analysis using cDNA microarray. The mRNA levels of several differentially expressed genes in cDNA microarray were further identified by Northern blot and RT-PCR.

RESULTS: A total of 190 differentially expressed genes including 151 IFN-α-repressed and 39 -stimulated genes or expressed sequence tags from 8 464 known human genes were found to be regulated by IFN-α in MHCC97. With a few exceptions, mRNA levels of the selected genes in RT-PCR and Northern blot were in good agreement with those in cDNA microarray.

CONCLUSION: IFN-α might exert its complicated anti-tumor effects on MHCC97 xenografts by regulating the expression of functional genes involved in cell metabolism, proliferation, morphogenesis, angiogenesis, and signaling.

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Key words: Interferon α; cDNA microarray; Gene expression profile; HCC

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INTRODUCTION

Human hepatocellular carcinoma (HCC) is one of the most prevalent malignancies in China. Patients with HCC often die of tumor metastasis and recurrence even after curative resection. Recently, a metastatic human HCC model in nude mice (LCI-D20) and a series of HCC cell lines (MHCC97, MHCC97-H, MHCC97-L) with different metastatic potentials derived from LCI-D20 have been established in our institute[1,2]. Using this model, IFN-α significantly inhibits tumor growth and metastasis of MHCC97 xenografts has been found[3-5]. However, the underlying molecular mechanisms are still unclear.

IFN-α is a multifunctional cytokine capable of interfering with viral infection, inhibiting cell proliferation, regulating cell differentiation, as well as modulating immune response[6-9]. It is well known that these pleiotropic effects of IFN-α are mediated primarily through the transcriptional regulation of many different functional genes. Thanks to the rapid progress in human genetic projects; many functional human genes and expressed sequence tags (ESTs) are identified and released, which make us possible to use cDNA microarray to survey IFN-α-modulated genes in MHCC97 cells. In this study, we identified 190 differentially expressed genes from 8 464 known human genes, which might mediate various biological functions of IFN-α. These data provide us useful clues for further studying the anti-tumor mechanisms of IFN-α and finding the IFN-α mimics for HCC therapy.

MATERIALS AND METHODS

Cell culture

MHCC97, a metastatic HCC cell line derived from LCI-D20 xenografts, was cultured in high glucose Dulbecco's modified Eagle's medium (Gibco-BRL, NY, USA) supplemented with 10% fetal calf serum (Hyclone, UT, USA), 100 U/mL penicillin and 100 μg/mL streptomycin in 20-cm^2 tissue culture flasks. Cells were grown at 37 °C in a humidified atmosphere of 50 mL/L CO_2 and passaged every 3 d.

cDNA microarray analysis

A total of 8 464 cDNAs of known human genes (United Gene Holding, Ltd, Shanghai) were amplified by polymerase chain reaction (PCR) using universal primers and spotted onto silylated slides (CEL Associates,

Houston, TX, USA) using a Cartesian PixSys 7500 motion control robot (Cartesian Tech, Irvine, CA, USA) fitted with ChipMaker micro-spotting technology (TeleChem, Sunnyvale, CA, USA). After being hydrated, dried, cross linked and washed, the microarray was ready for use. Total RNA was isolated from IFN-α-treated and untreated (3 000 IU/mL, 16 h) cells using TRIzol (Gibco-BRL). cDNA probes were prepared by reverse transcription and purified according to the methods described by Schena *et al*[10]. Then equal amount of cDNA from IFN-α-untreated and treated MHCC97 cells was labeled with Cy3-dUTP and Cy5-dUTP, respectively. The mixed Cy3/Cy5 probes were purified and dissolved in 20 μL of hybridization solution (0.75 mol/L NaCl, 0.075 mol/L sodium citrate, 0.4% SDS, 50% formamide, 0.1% Ficoll, 0.1% polyvinylpyrrolidone and 0.1% BSA). Microarrays were pre-hybridized with 0.5 mg/mL salmon sperm DNA at 42 °C for 6 h. After being extensively washed, the denatured (95 °C, 5 min) fluorescent-labeled probe mixture was applied onto the pre-hybridized chips and further hybridized at 42 °C for 15-17 h under a cover glass. Subsequently, chips were sequentially washed for 10 min at 60 °C with 2×SSC+0.2% SDS, 0.1×SSC+0.2% SDS and 0.1×SSC solutions and dried at room temperature (1× SSC: 150 mmol/L NaCl, 15 mmol/L sodium citrate). Both Cy3 and Cy5 fluorescent signals of hybridized chips were scanned by ScanArray 4000 (GSI Lumonics, MA, USA) and analyzed using Genepix Pro 3.0 software (BioDiscovery Inc., CA, USA). To minimize artifacts arising from low expression, only genes whose Cy3 and Cy5 fluorescent intensities were both over 200 counts, or genes whose Cy3 or Cy5 fluorescent intensity was over 800 were selected for calculating the normalization cofactor (ln(Cy5/Cy3)). Genes were identified as differentially expressed, if the ratio of Cy5/(Cy3×normalization cofactor) (Cy5/Cy3*) was more than 2 or less than 0.5.

Reverse transcription and polymerase chain reaction

MHCC97 cells (106) cultured in 20-cm^2 flasks were treated with 3 000 IU/mL IFN-α (Roche, Shanghai) for 0 or 16 h, and total RNA was extracted (RNeasy Mini Kit, QIAGEN Inc., CA, USA). One microgram RNA was used to set-up reverse transcription reactions (Gibco-BRL, NY, USA). Nine differentially expressed genes identified by cDNA microarray were selected for analysis by semi-quantitative PCR. Appropriate primers were designed using Primer3 software (http://www-genome.wi.mit.edu). γ-Actin was used as an internal standard. PCR reaction conditions and primer sequences are summarized in Table 1.

Northern blot analysis

Total RNA of 3 000 IU/mL IFN-α-treated or untreated MHCC97 cells was isolated as described above. Thirty microgram was separated by 1% agarose formaldehyde gel electrophoresis and transferred to a nylon membrane (Millipore, MA, USA) in 10×SSC by capillary blotting. The membrane was hybridized with the appropriate cDNA probe prepared from the human library of cDNA clones (Biostar Genechip Inc., Shanghai) and labeled with [α-^{32}P]dCTP (Yahui Biomedical, Beijing) using random primer (Ambion Inc., Austin, TX, USA).

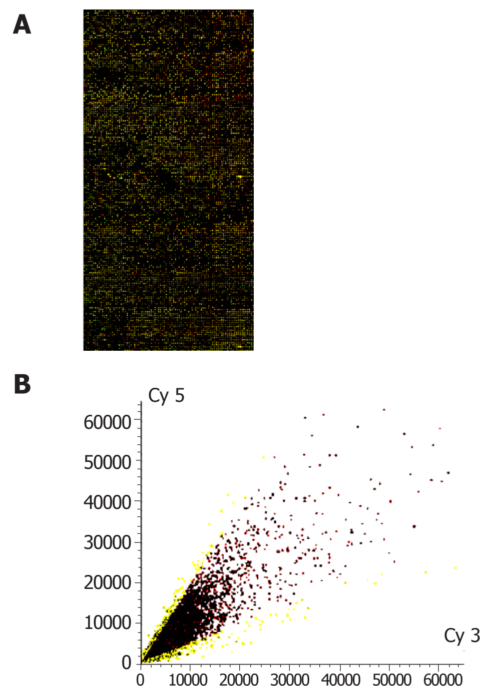

Figure 1 Representative hybrid result (**A**) and scatter plots (**B**) of cDNA microarray analysis in IFN-α treated MHCC97.

RESULTS

Gene expression profile identified by cDNA microarray

It is well known that the gene expression pattern of cells often varies with time and differentiation status and that cells derived from different individuals often have different genetic expression profiles. As a result, it is often difficult to extract useful information on the possible causes of phenotypic differences by comparing the genetic expression profiles of different cell lines. To minimize such complicated factors, we compared the gene expression profiles in 3 000 IU/mL IFN-α-treated and untreated (0 IU/mL) MHCC97 cells in two independent cDNA microarray analyses. We reasoned that such an internally consistent comparison might provide useful information on explaining the anti-tumor molecular mechanism of IFN-α in MHCC97 xenografts.

In 8 464 tested genes and ESTs, 190 genes were identified to be modulated by 3 000 IU/mL IFN-α treatment in MHCC97 cells. Among them the ex-pression of 151 genes was downregulated by IFN-α and the expression of 39 genes was upregulated by IFN-α. All differentially expressed genes are listed in Table 2 and the gene expression profiles obtained by cDNA microarray analysis are shown in Figure 1.

Nine differentially expressed genes evaluated by RT-PCR and Northern blot

To validate the results of cDNA microarray, we selected

Table 1 Primer sequence and condition for PCR analysis of selected genes

Category	Gene	Sense and antisense primers	Annealing (°C)	Cycles	Size (bp)
Cytoskeletal gene	Neutral calponin	5′-TGGCACCAGCTAGAAAACCT-3′; 5′-CAGGGACATGGAGGAGTTGT-3′	56	26	498
Proliferative gene	hMCM2	5′-ACCGAGACAATGACCTACGG-3′; 5′-CTAGCTGTCTGCCCCTTGTC-3′	56	30	382
Angiogenic gene	VEGF165 receptor	5′-GAAGCACCGAGAGAACAAGG-3; 5′-CACCTGTGAGCTGGAAGTCA-3′	56	30	359
IFN-α-induced genes	9-27	5′-TTGGTCCCTGGCTAATTCAC-3′; 5′-ATGAGGATGCCCAGAATCAG-3′	53	35	491
	ISG-56 ku	5′-AAAAGCCCACATTTGAGGTG-3′; 5′-GGCTGATATCTGGGTGCCTA-3′	54	30	451
MAPK pathway-related genes	ERK activator kinase (MEK2)	5′-CGAAAGGATCTCAGAGCTGG-3′; 5′-GTGCTTCTCTCGGAGGTACG-3′	56	26	349
	G3BP2	5′-GCAGAACCTGTTTCTCTGCC-3′; 5′-CACCACCACCTCTGGTTTCT-3′	56	30	475
	CHED	5′-TCCTTGGCGAACTCTTCACT-3′; 5′-TGCCATAAAGGGAGATCTGG-3′	56	30	336
cAMP/PI3 pathway-related gene	Adenylyl cyclase	5′-CCAGGAGCCTGAAGAATGAG-3′; 5′-GGCTTCTGAGCTCCAATCAC-3′	53	35	439
Housekeeping gene	γ-Actin	5′-ATGGAAGAAGAAATCGCCGC-3′; 5′-ACACGCAGCTCGTTGTAGAA-3′	55	25	287

nine genes whose expressions were clearly altered by IFN-α and evaluated their expressions by PCR and Northern blot. We enrolled IFN-α-regulated genes and found that the results were consistent with the previous reports[11,12].

For PCR analysis, we synthesized primers as indicated in Table 1 and performed semi-quantitative RT-PCR as outlined under "Materials and methods" after treatment of MHCC97 cells with 3 000 IU/mL IFN-α for 0 or 16 h. The transcription patterns of the same genes were also analyzed by Northern blot. Among the nine selected genes, seven downregulated genes were proved by cDNA microarray, six by RT-PCR and five by Northern blot analysis. Two stimulated genes, ISG-56 ku and 9-27 were proved by cDNA microarray, RT-PCR and Northern blot analysis. ERK activator kinase (MEK2), one repressed gene in cDNA microarray, was not changed in RT-PCR or Northern blot analysis. Thus, with a few exceptions, the results of RT-PCR and Northern blot were in good agreement with those of cDNA microarray analysis (Figure 2).

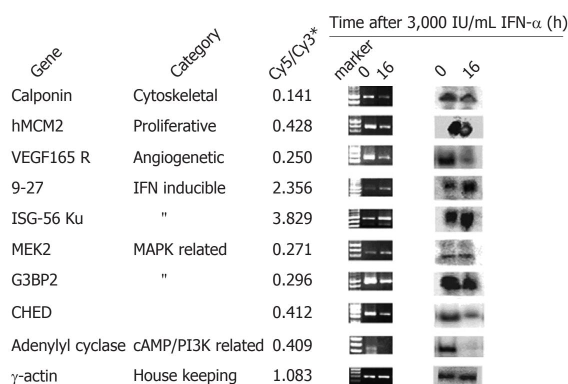

Figure 2 Confirmation of gene expression profiles in cDNA microarray analysis with RT-PCR and Northern blot.

DISCUSSION

cDNA microarray is a useful technique for rapid screening of gene expressions in cells, although the results need to be further confirmed by other molecular methods. Using this method, we found 211 hybrid dots, whose Cy5/Cy3* ratio was either more than 2 or less than 0.5 in IFN-α-treated MHCC97. Blasting the cDNA sequences in public database showed that these dots represented 190 different human genes or ESTs due to the redundant hybrids. Based on the results of RT-PCR and Northern blot, we believe that our cDNA microarray data are reliable. These differentially expressed genes might mediate the multiple biological functions of IFN-α directly or indirectly in MHCC97. We have artificially categorized these genes into nine functional clusters (Table 2).

IFN-α might interfere with cellular metabolisms by downregulating metabolic gene expression. In detail, IFN-α can inhibit glycolysis, glycogen degradation, gluconeogenesis as well as creatine or glucose transportation by repressing the expressions of liver-type phosphofructokinase (hPFKL), M2-type pyruvate kinase, brain glycogen phosphorylase, 2-oxoglutarate dehydrogenase, glucose transporter glycoprotein (SGLT) and cytosolic thyroid hormone-binding protein[13]. IFN-α can also inhibit lipolysis by reducing the expression of delta7-sterol reductase and pristanoyl-CoA oxidase, two key enzymes in lipid metabolism[14,15]. In addition, IFN-α reduces purine and pyridine biosynthesis by repressing the expression of GARs-AIRs-GART and serine hydroxymethyltransferase 2 (SHMT2). All these indicate that IFN-α-treated MHCC97 can result in lower ATP production and DNA synthesis, and slow down cell proliferation.

Many proliferation-, apoptosis- and cell cycle-regulating genes are modulated by IFN-α in MHCC97.

Table 2 Gene expression profile of MHCC97 cells induced by IFN-α

Category	GenBank ID	Gene description	Cy5/Cy3* (average)
2.1 Metabolism related genes	HUMCRTR	Creatine transporter	0.251
	HSGAGMR	GARs-AIRs-GART	0.289
	HUM2OGDH	2-Oxoglutarate dehydrogenase	0.298
	AF034544	Delta7-sterol reductase	0.318
	HUMTK	Thymidine kinase	0.333
	HSU12778	Acyl-CoA dehydrogenase	0.341
	HUMTHBP	Thyroid hormone-binding protein(p55)	0.349
	AF067127	7-Dehydrocholesterol reductase (DHCR)	0.356
	HSPRCOX	Pristanoyl-CoA oxidase	0.364
	AF035429	Cytochrome oxidase subunit 1	0.372
	AF070544	Glucose transporter glycoprotein (SGLT)	0.379
	HSPFKLA	Liver-type1-phosphofructokianse (PFKL)	0.392
	HUMSHMT	Serine hydroxymethyltransferase 2 (SHMT2)	0.407
	HUMMGPHB	Brain glycogen phosphorylase	0.413
	HUMTCBA	Cytosolic thyroid hormone-binding protein (p58)	0.415
	D88152	Acetyl-coenzyme A transporter	0.451
	HUMPKM2L	M2-type pyruvate kinase	0.456
	HSLDHBR	Lactate dehydrogenase B	2.156
	AF108211	Inorganic pyrophosphatase	2.25
	HSCOXVII	Cytochrome C oxidase VII	2.279
	HUMCYCPSK	Cytochrome C (HS7)	2.574
	HUMDBI	Diazepam binding inhibitor	2.628
2.2 Proliferation, apoptosis and damaged DNA repairing related genes	HSATPBR	Na/K ATPase beta subunit	0.208
	HUMP53T	Mutant p53 protein	0.233
	HSMITG	Mitochondrial DNA	0.309
	HSNUMAMR	Nuclear mitotic apparatus protein	0.325
	HSDNALIG3	DNA ligase III	0.34
	G28520	STS HSGC-31478 (homolog to Rad23a)	0.341
	AF096870	Estrogen-responsive B box protein	0.352
	AF001609	EXT like protein 3	0.367
	AF015283	Selenoprotein W	0.369
	AF011905	Putative checkpoint control protein hRad1	0.398
	HUMHMAM2	Minichromosome maintenance 2	0.408
	HUMRNAPII	RNA polymerase II 23 ku subunit	0.408
	AF007790	Inversely correlated with estrogen receptor Expression (ICERE-1)	0.413
	HSU78310	Pescadillo	0.43
	AF004162	Nickel-specific induction protein (Cap43)	0.434
	HSU3298	UV-damaged DNA binding factor	0.437
	HUMP1CDC47	P1cdc47	0.442
	HSU72649	B cell translocation gene 2	0.444
	AF031523	bcl-xL/bcl-2 associated death promoter (BAD)	0.481
	AF132973	CGI-39 (homolog to GRIM-19)	2.079
2.3 Morphogenesis, adhesion, and cytoskeleton remodeling related genes	D38735	Neutral calponin	0.141
	AF006082	Actin-related protein Arp2	0.197
	U01244	Fibulin 1D	0.212
	AF070593	Beta tublin	0.236
	HSU35622	EWS-E1A-F chimeric protein	0.255
	AF049259	Keratin 13	0.335
	HSPRO4HY	Prolyl 4-hydoxylase beta	0.337
	HUMCN4GEL	Collagenase type IV	0.36
	AF005654	Actin-binding double zinc-finger protein	0.378
	HSTEST	Testican	0.379
	HUMEPSURAN	Surface antigen	0.389
	AF004841	CAM-related/down-regulated by oncogenes	0.398
	HUMGLBA	Co-beta-glucosidase	0.402
	HUMCA1XIA	Alpha-1 type XI collagen	0.423
	HUMMCPGV	Macrophage capping protein	0.461
	HUMNID	Nidogen	0.497
	HSTUMP	Translationally controlled tumor protein	2.022
2.4 Signal transmitting related genes	HUMEPHT2R	Protein tyrosine kinase (NET PTK)	0.248
	HUMMEK2NF	ERK activator kinase (MEK2)	0.271
	HUMBADPTA	Beta-adaptin	0.273
	HUMP2A	Alpha-PR65	0.282
	HUMHRGAA	rab GDI alpha	0.285
	AF053535	ras-GAP/RNA binding protein G3BP2	0.296
	HSRING3GE	RING 3	0.316
	HSU45973	Pt Ins (4,5) P(2) 5-phosphatase	0.324
	HSU07139	Voltage-gated calcium channel beta	0.329
	HUMFTPB	Farnesyl-protein transferase beta	0.345
	HSU33053	Lipid-activated protein kinase (PRK1)	0.352
	HUMHK1A	Calcium-ATPase (HK1)	0.386
	HSU66406	EPH-related PTK receptor ligand LERK-8	0.386
	HSPP15	Placental protein 15	0.387
	HSADCYCL	Adenylyl cyclase	0.409
	HUMCHED	cdc2-related protein kinase (CHED)	0.412
	AF093265	Homer 3	0.415
	HSU40282	Integrin-linked kinase	0.416
	HUMGKAS	Stimulatory G protein	0.416
	HSU43939	Nuclear transport factor 2	0.429
	HUMCAK	Tyrosine protein kinase (CAK)	0.439
	HUMGNOS48	Endothelial nitric oxide synthase	0.443
	HUMCDPKIV	Calmodulin-dependent protein kinase IV	0.449
	HSPKX1MR	Protein kinase, PKX1	0.469
	D83760	Mother against dpp (Mad) related protein	0.472
	HUMEGFGRBA	EGF receptor binding protein GRB2	0.481
	HSU51004	Protein kinase C inhibitor (PKCI-1)	2.223
2.5 Tumor angiogenesis related genes	HUMRNAMBPE	Golli-mbp	0.236
	AF016050	VEGF 165 receptor/ neuropilin	0.25
	AF001307	Aryl hydrocarbon receptor nuclear translocator	0.27
	HSU64791	Golgi membrane sialoglycoprotein MG 160	0.355
	HUMPTPRZ	Protein tyrosine phosphatase Zeta-polypeptide	0.363
	HSU28811	Cysteine-rich FGFR (CFR1)	0.414
	HSU20758	Osteopontin	2.193

	HUMTR107	DNA-binding protein, TAXREB107	2.24
	HUMNEPPON	Nephropontin	2.413
2.6 Transcriptional activity related genes	S66431	Retinoblastoma binding protein 2	0.182
	HUMANT61K	Medium antigen-associated 61 ku protein	0.183
	HSU58197	Interleukin enhancer binging factor 2	0.226
	HSUBP	Upstream binding factor	0.266
	4758315	ets-related molecule, ETV5	0.267
	AF099013	Glucocorticoid modulatory element binding protein 1	0.309
	HSU72621	Lost on transformation 1(LOT1)	0.313
	HUMFOS	Oncogene protein, c-fos	0.361
	AB019524	Nuclear receptor co-repressor	0.369
	HS14AGGRE	Conserved gene telomeric to alpha globin cluster	0.398
	HSU74667	tat interactive protein (tip60)	0.404
	AF114816	KRAB-zinc finger protein SZF1-1	0.406
	HSU80456	Drosophila single-minded, SIM2	0.409
	AF117756	TRAP 150	0.41
	HSU15306	Cysteine rich DNA binding protein NFX1	0.417
	S57153	Retinoblastoma binding protein 1	0.469
	HUM56KDAPR	IEF SSP 9502	2.183
	HUMTR107	DNA binding protein. TAXREB 107	2.24
	HUMMSS1	Mammalian suppressor of sgv 1, MSS 1	2.313
2.7 mRNA and protein processing, secretory, proteolysis related genes	HSU39412	Alpha SNAP	0.141
	HSU47927	Isopeptidase T (ISOT)	0.229
	HSU72355	hsp27 ERE-TATA bind protein, HET	0.231
	AF077039	TIM17 homolog	0.238
	HUMHRH1	RNA helicase, HRH1	0.251
	AF206402	U5 SnRNP 100 ku protein	0.255
	D85429	Heat shock protein 40	0.344
	HSU85946	hSec 10p	0.378
	HSY10806	Arginine methyltransferase	0.412
	AB002135	Glycophosphatidylinositol anchor attachment 1	0.428
	AB007510	PRP8 protein	0.436
	HSU24105	Coatomer protein (COPA)	0.455
	HSCANPX	Calpain-like protease (CANPX)	0.456
	HSRBPRL7A	Ribosomal protein L7	2.067
	D89678	A+U-rich element RNA-binding protein	2.069
	HSU14966	Ribosomal protein L5	2.113
	HSRPL31	Ribosomal protein L31	2.142
	HUMPSC9	Proteasome subunit HC9	2.179
	HSU26312	Heterochromatin protein HP1 HS-gamma	2.182
	HUMRPS7A	Ribosomal protein S7	2.289
	AF106622	TIM17a	2.312
	HSUCEH3	Ubiquitin-conjugated enzyme UbCH2	2.323
	HUMRPS7A	Ribosomal protein S7	2.289
	AF106622	TIM17a	2.312
	HSUCEH3	Ubiquitin-conjugated enzyme UbCH2	2.323
	HUMRPS25	Ribosomal protein S25	2.326
	HUMRPSA3A	Ribosomal protein S3a	2.328
	HSRNASMG	Sm protein G	2.334
	HUMRPS18	Ribosomal protein S18	2.341
	HUMRP4SX	Ribosomal protein S4 isoform	2.346
	HUMPSC3	Proteasome subunit HC3	2.368
	HUMTCP20	Chaperonin protein, TCP20	2.572
	4504522	Chaperonin protein, hsp10	2.686
2.8 Tumor antigen processing, anti-viral infection related genes	HUMSAPC1	Cerebroside sulfate activator protein	0.211
	AF077011	Interleukin 16	0.23
	AF057307	Prosaposin	0.26
	HUMSIATA	Sialyltransferase	0.26
	AF055008	Epithelin 1 and 2	0.363
	HSU58766	FX protein	0.393
	HUMOSF1	OSF1	0.407
	HSU46194	RAGE 4	0.43
	HSU18121	136 ku double-stranded RNA binding protein	0.469
	AF021315	Reverse transcriptase	0.483
	S74095	Preproenkephalin A	2.115
	HUM927A	Interferon inducible protein 9-27	2.356
	HSIFI56R	Interferon inducible protein 56 ku	3.829
	HUMHCAMAP1	Interferon inducible protein 44 ku	4.03
2.9 Genes with unknown biological functions	D50928	KIAA0138	0.23
	AF132942	CGI08	0.269
	AB020677	KIAA0870	0.271
	AB011110	KIAA0538	0.277
	AB028956	KIAA1033	0.28
	HSU10362	GB36b glycoprotein	0.335
	4579277	A homolog of proteasome regulatory S2	0.352
	AB002356	KIAA0358	0.371
	4505130	A homolog of MCM3	0.371
	AB029020	KIAA1097	0.381
	HS130N43		0.383
	HSU66406	Eplg8	0.386
	HSNIPSNA1	NIPSNAP1 protein	0.391
	AB002378	KIAA0380	0.405
	HSU90907	Regulatory subunit of P55 PIK	0.407
	AB208959	KIAA1036	0.414
	AB020658	KIAA0851	0.416
	AF035282		0.416
	AF000136		0.419
	HUMORFFA	KIAA0120	0.424
	D13699	KIAA0019	0.43
	HUMORFB1	KIAA0123	0.432
	AF151830	CGI72	0.436
	AB007900	KIAA0440	0.437
	AB014595	KIAA0695	0.439
	HSM800064		0.439
	HUMORFA04	KIAA0115	0.457
	HSU79287		0.462
	AF007149		0.473
	AF007135		2.147
	AF151875	CGI117	2.184
	AF151857	CGI99	2.326
	HUMRSC508	KIAA0020	2.45

Downregulating the expression of mutant p53, mitochondrial DNA, nuclear mitotic apparatus protein (NuMA), and RNA polymerase II 23 ku subunit (polR2) might cause cell cycle arrest[16,17]. Downregulating the expression of DNA ligase III, hRad1, minichromosome maintenance 2 (hMCM2) as well as UV-damaged DNA binding factor might hinder damaged DNA repairing[18,19]. Stimulating retinoid-IFN-induced mortality 19 (GRIM-19) expression might promote IFN-α-induced apoptosis[20].

Several genes functionally related to cell morphogenesis,

adhesion, and cytoskeleton remodeling are also modulated by IFN-α in MHCC97. For example, decreasing the expression of calponin, actin-related protein 2 (Arp2), fibulin 1D, beta-tublin and epidermal surface antigen (ESA), *etc*., might damage mitotic spindle formation and might interfere with actin-based cell motility, migration, adhesion and morphogenesis[21-24]. Reducing the expression of prolyl 4-hydroxylase beta, a key enzyme in collagen biosynthesis and type IV collagenase, a tumor-derived extracellular matrix metalloproteases might block tumor invasion and metastasis. Although most genes in this category were first identified as IFN-α regulating genes, their roles in mediating IFN-α functions need to be further studied.

In this study, we found that many genes functionally related to signal transmitting were affected by IFN-α in MHCC97. By repressing the expressions of discoidin domain receptor, integrin-linked kinase, EPH-related tyrosine kinase (EPT2) and MEK2, *etc*., IFN-α might block cellular signaling initiated by tyrosine-kinase receptors[25,26]. By modulating the expressions of Rab GDI, Ras-related GTP-binding proteins and farnesyl-protein transferase and nuclear transport factor (NTF2) and G3BP2, a Ras-GAP/RNA binding protein, IFN-α might interfere with GTP/GDP exchange and nuclear import, thus influencing the recycles and activities of ras and its homologs[27-29]. By attenuating the expressions of adenylyl cyclase (AC) and phosphatidylinositol 4,5-bisphosphate 5-phosphatase (PtdIns (4,5)P(2)5- phospharase), a catalyzer of phosphatidylinositol 4,5-bisphosphate and PRK1, IFN-α might decrease inositol polyphosphate levels in cytosol and might inhibit the serine/threonine-kinase activities through cAMP/ PI3P signal pathway[30,31]. All these changes might exert inhibitory effects of IFN-α on MAPK and PI3K signaling. In addition, other signaling pathways such as Ca(2+), NO and TGFβ/hMAD-dependent signaling pathways are suppressed by IFN-α as well[32,33]. Plausibly Jak/STATs pathway, the most important IFN-α signaling pathway, is confirmed not to be regulated in IFN-α-treated MHCC97. The deficient expression of p48 (ISGF3γ) in this cell line may be the possible mechanism for the non-response of IFN-α priming via Jak/STATs pathway (data not shown).

In this study, we found that many angiogenic-related genes were regulated by IFN-α. By attenuating the expressions of Golli-MBP[34], VEGF 165 receptor and aryl hydrocarbon receptor nuclear translocator (ARNT)[35] as well as Golgi membrane sialoglycoprotein MG 160, a bFGF binding protein and cysteine-rich FGF receptor (CFR-1)[36], IFN-α may destroy the balance between pro- and anti-angiogenic factors and exert its inhibitory effects on tumor angiogenesis.

It is well known that cells usually respond to various stimuli by rapidly shifting the functions of transcriptional factors. Using this strategy, IFN-α might impose its anti-proliferative functions and hormone response by fluctuating the expression of several transcriptional factors or their cofactors such as retinoblastoma binding protein2 (RBP2), interleukin enhancer binding factor 2, lost on transformation 1 (LOT1) and KRAB-zinc finger protein (SZF1)[37-40].

In addition, IFN-α might hinder with mRNA/rRNA spicing and maturation by downregulating RNA helicase (HRH1), U5 snRNP[41] and affect protein transportation, secretion and proteolysis by downregulating alpha SNAP, GPAA1, hSec10p, hsp40 and isopeptidase T, a putative molecular in ubiquitin–proteasome pathway[42-44]. Meanwhile IFN-α might evoke anti-viral or tumor immune response by upregulating 9-27, 56 ku protein and p44 expressions.

Except for functionally definite genes, many ESTs with unknown functions were identified as IFN-α-regulated genes in our study (Table 2). In conclusion, cDNA microarray is a useful, rapid method for screening transcriptome of cells and potentially paves a way for elucidating IFN-α effects on tumor growth and metastasis.

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