IMMUNOLOGY ORIGINAL ARTICLE

Identification and characterization of the interferon- β -mediated p53 signal pathway in human peripheral blood mononuclear cells

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doi:10.1111/j.1365-2567.2009.03104.x Received 27 January 2009; revised 4 March 2009; accepted 12 March 2009. Both authors were closely involved in the design and execution of the experiments and in the writing of the manuscript. Correspondence: S. Sriram, Department of Neurology, Vanderbilt University Medical Center, 2201 Children's Way, Nashville, TN 37212, USA.

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Summary

The relationship between the p53 signal pathway and the response of human peripheral blood mononuclear cells (PBMC) to interferon (IFN)- β has hitherto not been examined. Using an oligonucleotide microarray, we found differential expression of at least 70 genes involved in the p53 signal pathway, including p53, which regulate cell proliferation and cell death following stimulation with IFN- β . We verified our observations on a limited set of p53-regulated genes at the transcriptional and translational levels. We also examined the consequences of the activation of the p53 signal pathway by IFN- β in PBMC. When cultured in the presence of T-cell mitogens, IFN- β restricted the entry of lymphocytes from the G0/ G1 phase to the S phase and reduced the number of cells in the G2 phase. The addition of IFN- β alone did not increase apoptosis. However, in the presence of actinomycin D, a DNA-damaging agent, addition of IFN- β enhanced the susceptibility of PBMC to apoptosis. These observations suggest that, in spite of the activation of a number of mutually overlapping pathways mediating cell death, cell cycle arrest was the most evident consequence of IFN- β signalling in PBMC.

Keywords: apoptosis; interferon- β ; cell cycle arrest; lymphocytes; p53

Introduction

Interferon (IFN)- β belongs to a family of naturally occurring molecules that have pleiotropic effects on immune and non-immune cells.^{1,2} The receptor for IFN- β is widely expressed in tissues, and the interaction of IFN- β with its receptor leads to oligomerization of the receptor and phosphorylation of the receptor-associated tyrosine kinases Janus kinase 1 (Jak1) and tyrosine kinase 2 (Tyk2). This then leads to the phosphorylation of signal transducers and activators of transcription 1 (STAT1) and STAT2, which subsequently dimerize, translocate to the nucleus and activate the transcription of a number of IFN-stimulated genes.^{3,4} Most of the type 1 interferonstimulated genes have IFN-stimulated response element (ISRE) sequences in the promoter region.^{5,6} Activation of the IFN-stimulated genes requires the binding of the activated STAT proteins with p48 to form a trimeric complex that is responsible for regulating IFN actions.

IFN- β is currently used as a therapeutic agent in the treatment of hepatitis induced by the hepatitis C virus, multiple myeloma and multiple sclerosis.^{7–9} In the three

major clinical applications of IFN- β , therapeutic benefits have in large part been derived from strategies focused on the proliferation and expansion of the target cells. Not surprisingly, a number of studies that examined the activation of genes by IFN- β have focused on the expression and regulation of proteins that mediate cell proliferation and apoptosis. These studies have shown increased expression of tumour necrosis factor (TNF), Fas ligand, and TNF-related apoptosis-inducing ligand (TRAIL) by IFN- β .^{10–14} IFN- β has also been shown to decrease the expression of Fas-associated death domain-like interleukin-1 β -converting enzyme inhibitory protein (FLIP) and immunosuppressive acidic protein (IAP), two proteins that inhibit apoptosis.^{15,16} Although the induction of death receptors and their ligands has been surmised to be one of the principal mechanisms of action of IFN- β , direct evidence of the role of IFN- β in cell proliferation and apoptosis in human lymphocytes is lacking. More recently, IFN- β was shown to increase the induction of p53, a key protein involved in the activation of apoptosis in murine fibroblasts; however, the response of human peripheral blood mononuclear cells (PBMC) to the effects of IFN- β and in

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Please cite this article in press as: Zhang F. and Sriram S. Identification and characterization of the interferon- β -mediated p53 signal pathway in human peripheral blood mononuclear cells, Immunology (2009) doi: 10.1111/j.1365-2567.2009.03104.x

particular activation of the p53 pathway remains unexplored. ^{14,17–20}

The tumour suppressor protein p53 is a key transcription factor that is involved in the regulation of cell proliferation and cell death.^{21,22} By preventing the proliferation of cells bearing damaged DNA, which if left unattended can lead to neoplasia, p53 facilitates repair of DNA and, if the damage cannot be repaired, it directs the cells towards apoptosis. Thus, p53 acts as a tumour suppressor, and this has been confirmed by the presence of mutations of the p53 gene in cancer.²³⁻²⁵ It was believed that IFN- β -mediated activation of p53 following viral infection of tumour cells would lead to rapid apoptosis before viral expansion could occur, and thus restrict viral spread.²⁶ This mechanism of action might explain the beneficial effects of IFN- β in the treatment of hepatitis caused by the hepatitis C virus.²⁷ The mechanism of activation of p53 and its downstream signal pathway in PBMC and their role in regulating autoimmune diseases, including multiple sclerosis, remain unknown.

In our study, for the first time, we set out to examine, in a cohort of normal healthy individuals, the expression patterns and functions of the genes involved in the p53 signal pathway following culture with IFN- β and their effects on lymphocyte survival. Such studies, we believe, are important for the following reasons: (i) a detailed study of the activation of the p53 signal pathway in the PBMC of healthy donors by IFN- β is currently lacking; (ii) understanding of the outcome of p53 activation of PBMC in vitro will provide a basis for recognition of p53 activation pathways in the PBMC of patients on treatment with IFN- β for viral or autoimmune diseases, and (iii) evidence of defects in the p53 activation pathway may allow the identification of patients who show sub-therapeutic responses to IFN- β .

We show that, despite the activation of a number of proteins that have pro-apoptotic functions by IFN- β , the predominant effect on cell division was the induction of cell cycle arrest, and not apoptosis. These novel results have implications for the mechanism of action of IFN- β in the regulation of lymphocyte function *in vivo*.

Materials and methods

Subjects

The study group comprised 12 healthy volunteers who had no history of autoimmune disease and were not on any immunotherapy. The male to female ratio was 1 : 1; the ages of the subjects ranged from 30 to 60 years. Human subject studies were approved by the Committee for the Protection of Human Subjects of the Vanderbilt University Institutional Review Board.

Reagents

The RNA isolation kit and RNAse-free DNAse set were from Qiagen (Valencia, CA). cDNA was generated using Reverse Transcription Reagents (Applied Biosystems, Foster City, CA), and the iQ SYBR Green Supermix was from Bio-Rad Laboratories (Hercules, CA). The following primary antibodies were obtained and used in the indicated dilutions: mouse anti-human p53 antibody (DO-1) (1:2000), rabbit anti-human p21 antibody (1:2000), rabbit anti-human Bcl-2-associated X protein (Bax) antibody (1:2000), rabbit anti-human STAT1 antibody (1:5000), rabbit anti-human STAT2 antibody (1:5000),rabbit anti-human *B*-actin antibody (1:10000), secondary horseradish peroxidase linked anti-mouse immunoglobulin (IgG) and anti-rabbit IgG (1:10 000); all these antibodies were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA). The CD3fluorescein isothiocyanate (FITC)-conjugated anti-human antibody and the Annexin V-FITC & 7-amino-actinomycin D (AAD) apoptosis detection kit were from BD Biosciences Pharmingen (San Jose, CA). DNAse-free RNAse and propidium iodide were from Roche Applied Science (Indianapolis, IN). IFN- β -1a was a gift from Serono Inc., (Rockland, MA). Actinomycin-D (Act D), phytohaemagglutinin (PHA) and Ficoll-Hypaque was purchased from Sigma-Aldrich (St Louis, MO).

Isolation and culture of PBMC

PBMC were isolated by density gradient centrifugation with Ficoll-Hypaque from freshly heparinized blood. The cells were washed in phosphate-buffered saline (PBS) and re-suspended at 1×10^6 cells/ml in complete RPMI-1640 medium containing 2 mmol glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% fetal bovine serum (Invitrogen, Carlsbad, CA). The induction of p53 was examined at the following doses of IFN- β : 100, 1000 and 5000 IU/ml. The PBMC were cultured in the presence of 10 µg/ml PHA and Act D was used at a single dosage of 50 ng/ml.

Total RNA extraction and reverse transcription

Total RNA was extracted from PBMC using the RNeasy mini kit (Qiagen, Valencia, CA) and treated with the RNAse-free DNAse set, following the manufacturer's recommendations. A Bioanalyzer microfluidic assay (Agilent Technologies, Palo Alto, CA) was applied to test RNA integrity. Spectrophotometric and fluorometric methods were combined to quantify RNA. cDNA was generated from RNA using Reverse Transcription Reagents (Applied Biosystems). One microgram of total RNA was reverse-transcribed in a total volume of 25 μ l using 100 units of reverse transcriptase, 2.5 μ l of 10 × reverse transcriptase.

scription buffer, $2.5 \,\mu$ l of $10 \times$ random primer and $1.5 \,\mu$ l of $20 \,U/\mu$ l RNase inhibitor. The mixture was incubated for 10 min at 25°, 120 min at 37° and 5 seconds at 85° and then rapidly cooled on ice. The cDNA samples were stored at -20° .

Microarray analysis

To determine the differentially expressed genes in PBMC following culture with IFN- β , we used the GeneChip[®] Human Gene 1.0 ST (Affymetrix Inc., Santa Clara, CA). This chip contains 764 885 probes representing 28 869 genes, each of which is represented on the array by approximately 26 probes spread across the full length of the gene. Peripheral blood mononuclear cells were obtained from five healthy individuals. The isolated PMBC were cultured with IFN- β (1000 IU/ml) for 0, 24 and 48 hr. RNA samples were submitted to the Vanderbilt Microarray Shared Resource (Vanderbilt University, Nashville, TN, USA) for microarray analysis using the GeneChip Whole Transcript (WT) Sense Target Labeling Assay protocol (Affymetrix Inc., Santa Clara, CA). Briefly, a total of 100 ng of total RNA was reverse-transcribed to cDNA which was then used as a template in an in vitro transcription reaction followed by fragmentation of the single-stranded cDNA and labelling through a terminal deoxy-transferase reaction. The biotinylated cDNA (5 µg) was fragmented and hybridized to the Human Gene 1.0 ST Array, which was then scanned using GENECHIP SCANNER 3000 7G Plus 2 and COMMAND CONSOLE Software (AGCC) version 1.0 (Affymetrix Inc.). Generated CEL files (raw Affymetrix data) were imported into EXPRESSION CONSOLE (Affymetrix Inc.) and normalized by robust multi-array average (RMA)-sketch for quality control purposes.²⁸ Normalized data were uploaded into PARTEK GENOMICS SUITES (Partek Inc., St Louis, MI) for statistical analysis. To identify significant differences in gene expression level among the groups, log₂ gene expression measurements for each gene on each chip were modelled using a multifactor mixed model in the PARTEK GENOMICS SUITES software. In order to increase sensitivity and allow identification of

Table 1. Primers for quantitative real-timereverse transcription-polymerase chain reaction(RT-PCR)

potentially important biological changes, we employed a lower level of stringency and set an adjusted *P*-value [false discovery rate (FDR)] cut-off of 0.2. The lists of differentially expressed genes were then classified according to their biological pathway and biological processes. This was achieved using the protein analysis through evolutionary relationships (PANTHER) Classification System to compare them with reference lists to look for enriched functional categories.²⁹

Real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR)

Real-time quantitative PCR was carried out in an iCycler detection system (Bio-Rad laboratories, Hercules, CA) in a volume of 25 µl. The reaction mixture consisted of 12.5 µl of iQ SYBR Green Supermix, 200 nM of each primer, and 1 µl of cDNA template. Reactions were performed for 45 cycles (95° for 15 seconds, 60° for 30 seconds and 72° for 30 seconds) after an initial 3-min incubation at 95°. Primers for the different genes amplified are shown in Table 1. The primers for p53 comprised regions that overlapped the full length and the beta/gamma isoform of p53. All reactions were performed in duplicate. Values for each gene were normalized to the values of the internal control β -actin using the threshold cycle (C_t) method, and the fold change compared with the culture control was calculated.

Western blot analysis

Cell lysates for western blotting were prepared by treating PBMC with 50 mm Tris (pH 8.0), 200 mm NaCl, 1% NP40 supplemented with 5 µg/ml aprotinin, 5 µg/ml leupeptin, 1 mm NaF, 20 mm β -glycerophosphate, 1 mm sodium vanadate, 1 mm dithiothreitol and 1 mm phenylmethysulphonyl fluoride. The cells were incubated on ice for 30 min and sonicated, before being centrifuged at 18 000 **g** for 15 min. The total protein concentration was measured according to the Bradford assay method (Bio-Rad Laboratories). Equal amounts of protein were loaded

	Forward primer	Reverse primer
p53	CGTCAGAAGCACCCAGGACT	CATCCTCCTCCCCACAACAA
p21	TCCTCTAGCTGTGGGGGTGA	GAAGGTCGCTGGACGATTTG
BAX	CAGCAAACTGGTGCTCAAGG	CGGAGGAAGTCCAATGTCCA
MDM2 ³⁵	CAAGTTACTGTGTATCAGGCAGGG	TCTGTTGCAATGTGATGGAAGG
NOXA	ACCGCTGGCCTACTGTGAAG	TGTGCTGAGTTGGCACTGAAA
PUMA	GACCTCAACGCACAGTACGAG	AGGAGTCCCATGATGAGATTGT
STAT1	TGCAAATGCTGTATTCTTCTTTGG	TATGCAGTGCCACGGAAAGC
STAT2	CCTGCTGTGCTGGGAGGTAT	GAAAGAAGCCACTGCCCTGA
β -actin	GCCGAGGACTTTGATTGCAC	TGGACTTGGGAGAGGACTGG

BAX, Bcl-2 associated X protein; MDM2, murine double minute 2; STAT, signal transducers and activators of transcription.

onto a 12% sodium dodecyl sulphate (SDS)-polyacrylamide gel in electrophoresis buffer (25 mM Tris-HCl, 250 mM glycine and 0.1% SDS) and separated at 100 V. Proteins were then transferred onto polyvinylidene difluoride membranes (Millipore Corporation, Bedford, MA) by electroblotting for 1 hr at 100 V. After blocking with blotto (1 × Tris buffer solution (TBS), 0.05% Tween-20 and 5% non-fat milk powder) for 2 hr, the membranes were probed with primary antibodies. After three washes, secondary horseradish peroxidase linked anti-mouse IgG or anti-rabbit IgG was added for 1 hr. Specific bands were visualized using enhanced chemiluminescence reagent and exposed to X-ray film. The intensity of the bands was quantified using WCIF IMAGE J software (Wright Cell Imaging Facility, Toronto, Canada.). The ratio of the intensity of the band of the tested protein and that of β -actin was measured on the same membrane.

Detection of apoptosis

Apoptosis was analysed by labelling with the Annexin V-FITC & 7-AAD apoptosis detection kit. PBMC were cultured with IFN- β for 48 hr in either the presence or absence of DNA-damaging agent Act D for 24 hr before harvesting. At the end of the culture period, the cells were washed and stained with Annexin V-FITC and 7-AAD, and then were submitted to the BD LSRII flow cytometer (BD Biosciences, San Jose, CA). Data were analysed using BD FACSDIVA software (BD Biosciences) and cell apoptosis was determined by Annexin V⁺ and 7-AAD⁻.

Cell cycle analysis

PBMC (2×10^6) were stained with CD3-FITC for 30 min, washed twice and fixed in 75% ethanol at 4° for 2 hr, and then washed in PBS and subjected to digestion with DNAse-free RNAse for 0.5 hr at 37°. Cells were re-suspended in 500 µl of PBS with propidium iodide, and then submitted to the BD LSRII flow cytometer. Flow cytometry data were analysed using FLOWJC software (FlowJo, Ashland, OR).

Statistic analysis

Results are expressed as mean \pm standard deviation. Statistically significant differences among groups were identified using analysis of variance (ANOVA). Specifically, we employed repeated measures ANOVA for the data obtained in the western blotting, real-time RT-PCR and cell cycle experiments. The PROC MIXED procedure in SAS (version 9.1; SAS Institute, Cary, NC) and the SIMULATE adjustment were used to compute adjusted *P*-values of all pairwise differences of three time point's measurements for each parameter such as p53, p21 and Bax. The data from apoptosis experiments were analysed using one-way ANOVA in SPSS 11.0 software (SPSS, Chicago, IL) and *P*-values < 0.05 were considered significant.

Results

Activation of the p53 signal pathway by IFN- β

We examined the genes involved in the p53 signal pathway that were targeted by IFN- β using GeneChip[®] Human Gene 1.0 ST. A list of 8060 genes that showed a statistically significant change from baseline (FDR < 0.2) was generated. Among the 74 genes that were recognized as being involved in the p53 signal pathway, apoptosis and the cell cycle were two of the most highly represented biological processes (Tables 2 and 3, Fig. 1a). Of these genes, 16 were involved in the cell cycle process, 15 in apoptosis and 10 in both (Table 3). As shown in the heat map (Fig. 1a), there was an increase in p53 expression in cells cultured with IFN- β . The previously recognized downstream targets of p53, such as p21, PUMA, NOXA, Bax and growth arrest and DNA damage inducible gene 45 (Gadd45), were all shown to be induced by IFN- β . Genes that regulate the expression of death receptor-associated genes, such as those belonging to the TNF superfamily [Fas-associated protein with death domain (FADD), TNF receptor-associated factor 2 (TRAF2), TNF, apoptosis stimulating fragment (FAS), Fas ligand (FASLG)] and those involved in the common apoptotic pathway (apoptosis inducing factor 2 and Caspase 9), were also up-regulated. We also noted increased expression of murine double minute 2 (MDM2), a protein known to down-regulate apoptosis by inhibiting the actions of p53.

To determine levels of p53 mRNA in cells cultured with IFN- β , we performed real-time RT-PCR on RNA isolated from the PBMC of seven individuals, which were cultured with 1000 IU/ml of IFN- β , using primers specific for p53, p21, PUMA, NOXA and MDM2. After 48 hr of culture with IFN- β , there were significant increases (P < 0.05) in the expression of p53 (2·3-fold), p21 (12-fold), PUMA (3·5-fold), NOXA (5·5-fold), MDM2 (5-fold) and Bax (2·8-fold), compared with PBMC cultured in medium alone. At 24 hr, only NOXA, p21 and MDM2 showed a statistically significant difference from 0 hr (Fig. 1b–g).

Induction of p53 and p53 targeted proteins was also examined using western blot assays. We examined the kinetics of induction of p53 following *in vitro* culture of PBMC with IFN- β from 12 healthy volunteers. The addition of 1000 U/ml IFN- β was sufficient for significant induction of p53 at 48 hr (Fig. 2a,b) and there was a time-dependant increase in the full-length and beta/ gamma isoforms of p53. The increase in protein level was already significant at 24 hr and increased further at 48 hr (P < 0.05 compared with baseline). Densitometric studies for 12 individuals showed a 1.92-fold increase in

Гable 2.	Genes in the	p53 s	ignalling	pathway	are	involved	in tl	ne response	e of	PBMC	to	interferon	(IFN)	-β
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Gene accession number P-value Definition FML NM, 02390 7-785-11 Promyelocytic leukaemia sequence 1 (BC12-related) BRCA2 NM, 00009 1-446-08 Freast cancer 2, early onset STA72 NM, 000140 1-446-08 Freast cancer 2, early onset STA72 NM, 000143 6-306-07 Freast cancer 2, early onset CDRNIA(p21) NM, 07467 7-475-07 Cyclin-dependent kinase inhibitor 14. (p21, Cp1) PAIP/LINX0A NM, 000873 3-396-06 Taberons secrets induced protein 1 IGF1R NM, 000874 1-115-65 Cyclin A1 TSC2 NM, 000874 1-115-65 Cyclin A1 STA1C NM, 000874 2-366-65 Protein kinkase, DNA-sciented, calchip tophyperglod STRT NM, 016538 2-387-65 Horos spicos sirtui (clear maring type information regulatine 2 homologue 1 STA1 NM, 016538 2-387-65 Taberons science indologue 3 (protein kinase 8, parmal) REKA NM, 01034 6-367-65 Taberons science indologue 3 (protein kinase 8, parmal) REKA NM, 001034 6-367-		GenBank		
PMLNML 0332407.287-11Promydcarlic leukaemiaMCL1NML 0319608.601-09Mycloid cell leukaemia sequence 1 (BCL2-related)RRCA2NML 0084191.441-07Signal transducer and activator of transcription 2, 113 kDaSTAT2NML 0084191.441-07Fis (TTNF receptor superdimily, remeber 6)CDKNL (Apr1)NML 0784677.47E-07Fis (TTNF receptor superdimily, remeber 6)CDKNL (Apr1)NML 008753.39E-06Insulin-like growth factor 1 receptorTSC2NML 0008753.39E-06Insulin-like growth factor 1 receptorCCNA1NML 0008753.39E-06Insulin-like growth factor 1 receptorCCNA1NML 0008762.68E-05Protein Kinase, DNA-activated, cathyler phyceptideCRNANML 0008702.68E-05Protein Kinase, DNA-activated, cathyler phyceptideSRT7NML 0165382.88E-05Protein Kinase, DNA-activated, cathyler phyceptideSRT7NML 0165382.68E-05vacit enticido growth consegue homologue 3 (protein kinase R, gamma)TSC1NML 0165386.3EF-05Tuberous sclerosis 1CD6747NML 018300.00012/07Beta2-microglobuliniTSC1NML 0003800.0002705Beta2-microglobuliniTSC2NML 0102300.0002705Beta2-microglobuliniTSC1NML 002390.00027452Protein Kinase, AMP-activated, gamma 2 non-catabil's cabunitTSC1NML 002390.00027452Protein Kinase, AMP-activated, gamma 2 non-catabil's cabunitTSC2NML 002390.0003297Protein	Gene	accession number	P-value	Definition
MCL1 NM_021960 8-607-09 Mydeloid cell lackaemia sequence 1 (BCL2-related) BRCA2 NM_000519 1-481-07 Signal transducer and activator of transcription 2, 113 kDa STAT2 NM_000543 6-307-07 For (TTS) receptor superform), remether 0 CDKN1A(p21) NM_078467 7-477-67 Cyclin-dependent kinase inhibitor 1A (p21, Cip1) PMAIPI(NOXA) NM_000548 1-127-06 Tuberous selerosis 2 COXAI NM_000548 1-127-06 Approximation indice provide indiced protein 1 FASL NM_000549 1-407-06 Approtein-indiced protein indiced protein 1 FASL NM_000548 1-217-06 Approtein-indiced protein indiced protein indiced protein indiced protein indiced protein associated, 2 PRKDC NM_000548 2-487-06 Protein kinase, DNA-activated, catalytic polypeptide RTA1 NM_01075 4-997-05 v-alt maring type information regulation 2-bomologue 3 (protein kinase B, gamma) RELA NM_01038 6-327-05 Tuberoos selerosis Comortein andiogype indice RELA NM_001208 0-00012/075 Beta-2-000014/011 NMD410120 NMO10120 <tr< td=""><td>PML</td><td>NM_033240</td><td>7·78E-11</td><td>Promyelocytic leukaemia</td></tr<>	PML	NM_033240	7·78E-11	Promyelocytic leukaemia
IBRCA2NML, 0000991-144-08Breast cancer 2, anly onstSIAT2NML, 0000436-308-07Signal transducer and activator of transcription 2, 113 kDaEASNML, 0000436-308-07Fea (TNF receptor superfamily, member 6)DKNL1/2012NML, 0784077-478-07PMAUPI(NOXA)NML, 0211272-211-06Phorbol-12-myrisT/X15-13-acetac-induced protein 1IGFIRNML, 0008753-397-06Insulin-like growth factor 1 receptorTSC2NML, 0008753-397-06Feal (gand TNF superfamily, member 6)ALPAQNML, 0027972-308-05Protein Kinase, DNA-acrivatice, clashytic phyreptidePKRUCNML, 006382-381-05Protein Kinase, DNA-acrivatice, clashytic phyreptideSRT7NML, 016382-381-05Vietomic thyronau viruin (silent mating type information regulation 2 homologue) 7PKRUCNML, 016382-381-05Vietomic thyronau viruin (silent mating type information regulation 2 homologue) 7RTANML, 016386-258-05Tuberos selencis 1SCINML, 0014306-0012705Ret-2-acrivation faitance and acrivators acrivated acrivaticators and acrivators and acrivated acrivaticators and acrivators acrivated acrivated, acrivated, acrivata, acrivata, acrivated, acrivated, acriv	MCL1	NM_021960	8.60E-09	Myeloid cell leukaemia sequence 1 (BCL2-related)
STAT2 NM_0004 640-7 Fas (INF recorts regrammly, member 6) EAS NM_00004 640-7 Fas (INF recorts regrammly, member 6) CDKNLA[p21) NM_00127 2211-66 Phorbal-12-myrISTATe-13-acctate-induced protein 1 DFIR NM_00053 359E-66 Insulin-like growth factor 1 receptor TSC2 NM_00057 239E-66 Apostical factor 1 receptor CANAI NM_00057 230E-66 Apostical factors, mitochondrion-associated, 2 PRKDC NM_00058 2480-65 Protein kinase, DNA-accivated, catalytic polypeptide SIRT7 NM_01058 2481-65 Home saplews stimic factor, mitochondrion-associated, 2 PRKDC NM_00058 2480-65 Protein kinase, DNA-accivated, catalytic polypeptide SIRT7 NM_00058 6251-65 Taberous selerosis 1 CatorrA SIRT NM_000408 04001-2075 Breta-arimodobulin MDM2 NM_002031 04002452 Protein kinase, Catalytic, polypeptide MDM2 NM_002031 040027546 Phosphoinsitidatakes, catalytic, polypeptide MMA000057 0400084067<	BRCA2	NM_000059	1·44E-08	Breast cancer 2, early onset
EAS NML 00043 6-30E-07 Fas (TNF receptor superfamily, member 6) CNRN1A(021) NML 021127 221E-06 Photbol-12-myriSTATc-13-acetate-induced protein 1 IGHR NML 000875 339E-06 Insulin-like growth factor 1 receptor TSC2 NML 000874 112E-05 Tobs rous sclerosis 2 CCNA1 NML 000914 113E-05 Cyclin A1 FASLG SML 000875 44005 Fas Iigand (TNF superfamily, member 6) ATTM NML 01638 283E-05 Apoptosis-inducing factor, mitochondrion-associatel, 2 PRKDC NML 01638 283E-05 I-memor superiors sirulan (callent mating type information regulation 2 hornologue) 7 ATT3 NML 01860 422E-05 -vale murine thymomos viral oncogene homologue A (avian) TSC1 NML 000086 625E-05 Tuberous sclerosis 1 C20mf74 NML 01203 00002735 Beta-3-microglobulin MDM2 NML 01203 00002735 Beta-3-microglobulin MST14 NML 01023 000027454 Protein kinase, Alm-Acadage-inducib, be ta RMDM2 NML 016035 000027456	STAT2	NM_005419	1.84E-07	Signal transducer and activator of transcription 2, 113 kDa
CDKN1A(p21) NM. 079407 7-47E-07 Cyclin-dependent kinase inhibitor 1A (p21, Gp1) HAMPI(NOXA) NM. 000875 339E-06 Insulin-like growth factor 1 receptor IGFIR NM. 000875 339E-06 Insulin-like growth factor 1 receptor TSC2 NM. 00089 1-12E-05 Taberous sclerosis 2 CANA1 NM. 000897 2-90E-05 Appotosi-inducing factor, mitochondrion-associated, 2 FAKJG NM. 000904 2-68E-05 Protein kinase, DNA-activated, catalytic polypeptide SIRT7 NM. 018109 4-22E-05 v-akt marine thymoma viral oncogene homologue 3 (protein kinase B, gamma) RELA NM. 021975 4-99E-05 v-rel rectulendotheliosis viral oncogene homologue 3 (protein kinase B, gamma) RELA NM. 020408 6-0002776 Retaamicrofobulin MDM2 NM. 0004084 6-000237465 Chromosome 20 open reading frame 74 REA NM. 0004031 0-00023745 Retaamicrofobulin MDM2 NM. 001633 0-00023745 Reta-microsoma) GADD45B NM. 001633 0-00023745 Reta-microsomicropic leakakeemia) 4 GADD4	FAS	NM_000043	6·30E-07	Fas (TNF receptor superfamily, member 6)
PMAPI (X0XA) NML (20127 2:31E-06 Phorbol-12-myrKTATe-13-accette-induced protein 1 ISFLR NML (000548 1:12E-05 Tuberous sclerosis 2 ISC2 NML (000548 1:12E-05 Tuberous sclerosis 2 CCNA1 NML (000548 1:12E-05 Tuberous sclerosis 2 CCNA1 NML (000548 1:12E-05 Protein kinas, NMA archivated, catalytic polypeptide ALTM2 NML (01538 2:83E-05 Protein kinas, NMA archivated, catalytic polypeptide SIRT7 NML (01058 2:83E-05 V-rait reticulandotheliosis viral oncogene homologue 3 (protein kinase, NJA archivated, catalytic polypeptide SIRT4 NML (01058 6:25E-05 V-rait murine thymomo viral oncogene homologue 4 (avian) TSC1 NML (00048 6:02E-05 V-rait murine thymomo viral oncogene homologue (mouse) TSC1 NML (00048 6:02E-05 Tuberous sclerosis 1 Catadyta C20dr74 NML (00048 6:02E-05 Tuberous sclerosis 1 Catadyta C30dr74 NML (01075 Bitadyta 2-bitadyta 2-bitadyta 2-bitadyta 2-bitadyta 2-bitadyta 2-bitadyta 2-bitadyta 2-bitadyta 2-bitadyta 2-bitadyta2-bitadyta2-bitadyta 2-bitadyta 2-bitadyta2-bitadyta 2-bitadyta 2	CDKN1A(p21)	NM_078467	7·47E-07	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)
IGFIR NM_000875 339E-66 Insulin-like growth factor 1 receptor TSC2 NM_00059 142E-65 Tuberous sclerosis 2 CCAN1 NM_003914 113E-05 Cyclin A1 FASLG NM_00597 230E-05 Approtosi-inducing factor, mitochondrion-associated, 2 PRKDC NM_00594 268E-05 Protein kinase, DNA-activated, catalytic polypeptide SIR17 NM_010584 248E-05 Hornes acplues siturin (silent mating type information regulation 2 homologue) 7 AKT3 NM_010484 643E-05 Chromosane 20 open reading fame 74 SIR17 NM_000348 643E-05 Chromosane 20 open reading fame 74 C20of74 NM_000348 643E-05 Chromosane 20 open reading fame 74 SIR1 NM_000348 640E-27 Reta 2-microglobulin MDM2 NM_00234 040012707 Reta 2-microglobulin MDM2 NM_00234 0400274852 Protein kinase, AMP-activated, gamma 2 non-catalytic subunit GADD458 NM_01675 040058462 Retionbalstoma 1 (including ostessarcom) PIKAG2 NM_000371 040058988	PMAIP1(NOXA)	NM_021127	2·21E-06	Phorbol-12-myriSTATe-13-acetate-induced protein 1
TSC2NM_0005481128-05Tuberous clerosis 2CCNA1NM_0005491438-05Cyclin A1FAGGNM_0005491408-05Fas ligand (TNF superfamily, member 6)AIFN2NM_0105392:30E-05Apoptosis-inducing factor, mitochondrion-associated, 2PKRDCNM_0105382:83E-05Protein kinase, DN-activated, catalytic polypeptideSIR17NM_0105382:83E-05V-act murine thymona viral oncogene homologue 3 (protein kinase B, gamma)RELANM_0219754:99E-05v-cl reticuloendotheliosis viral oncogene homologue 4 (avian)TSC1NM_000866:23E-05Tuberous sclerosis 1Codorf/4NM_000876:000127075Beta-2-microglobulinDDAZOb00127075Beta-2-microglobulinmonologue (mouse)MYSI4NM_0102300:000234052Rotholk protein kinase, AMP-activated, gamma 2 non-cutalytic subunitPKAC2NM_0102030:000234052Rotholk protein kinase, AMP-activated, gamma 2 non-cutalytic subunitRBINM_000310:00034028Retinoblatoma 1 (including osteosarcoma)PKAC2NM_0008710:00034028Retinoblatoma 1 (including osteosarcoma)PKAC3NM_0001800:000980872Insulin-like growth factor 1 receptorHK2NM_001890:00029845Retinoblatoma 1 (including osteosarcoma)PKAC2NM_001890:0012907RCL2 binding component 3GARDLINM_001890:00029845Retinoblatione 2PKAC2NM_001890:0012907RCL2 binding cortein 1.3RFANM_001	IGF1R	NM_000875	3·39E-06	Insulin-like growth factor 1 receptor
CCNA1NML.0039141-13E-05Cyclin A1FASLGNML.0006391-40E-05Fas ligand (TNF superfamily, member 6)AIMA2NML.0207972:30E-05Apoptosis-inducing factor, mitochondrion-associated, 2PRKDCNML.0069042:68E-05Protein kinase, DNA-activated, catalytic polyperpideSIR17NML.016582:88E-05Homes appins strintic (slet mating tyre information regulation 2 homologue) 7AKT3NML.0219754:99E-05v-act matine thymoma viral oncogene homologue 4 (avian)TSCLNML.003686:25E-05Tuberous sclerosis 1C200rf74NML.003686:25E-05Chromosome 20 open reading fame 74BZMNML.003750:00012707BE42microglobulinMDML2NML.003750:00012707BE42microglobulinMDML2NML.003750:000278463Growth arrest and DNA-damage-inducible, betaPRKAG2NML.0162030:000278452Protein kinase 13GADD458NML.0027540:00054056Hoopshonisitide-3-kinase, catalytic, beta polypeptideMARI3NML.0027540:00054057Insulin-like growth factor 1 receptorHK2NML.001790:000598988Mitogen-activated protein kinase 13GCSI/UMA)AE365450:0013523Capase 4:aviated fortor 1PCNANML.002590:0013524Roline ereptorHK2NML.001590:0013525Nuclear factor of activated 7-cells, reptalseCINANML.002590:0013525Nuclear factor of activated 7-cells, reptalsePCNANML.002590:00135	TSC2	NM_000548	1·12E-05	Tuberous sclerosis 2
FASLGNA_0005391-40E-05Fas lgand CNP superfamily, member 6)AIFM2NM_0327972-30E-05Approxais-inducing factor, mitochondron-associated, 2PREDCNM_0069042-68E-05Protein kinase. DNA-activated, catalytic polypeptideSIRT7NM_016582-82E-05v-skt murine thymoma viral oncogene homologue 3 (protein kinase B, gamma)RLIANM_0219754-99E-05v-rel reticuloendotheiosis viral oncogene homologue 4 (avian)SIRT6NM_0203436-63E-05Chromosome 20 open reading fame 74Codorf74NM_0004840-000127075Beta-2-microglobulinDMDANM_0023300-00023403Growth arrest and DNA-damage-inducible, betaREXNM_0123300-00023403Growth arrest and DNA-damage-inducible, betaPRKAG2NM_0162300-00023462Protein kinase, AMP-activated, gamma 2 non-catalytic subunitRB1NM_000310-00034028Ritinoblastoma 1 (including osteosarcoma)PRKAG2NM_0018700-00059868Mitogen-activated protein kinase 13RB1NM_0027510-00059868Mitogen-activated protein kinase 13RB1NM_0027500-00059868Mitogen-activated factor 2PCNANM_001890-0012607RDL2 binding component 3GARN1NM_002890-0012607RDL2 binding component 3GARN1NM_012810-0012607RDL2 binding component 3GARN1NM_012890-0012607RDL2 binding component 3GARN1NM_012890-0012607RDL2 binding cortein 2,48 KDaRAFC2 <td>CCNA1</td> <td>NM_003914</td> <td>1·13E-05</td> <td>Cyclin A1</td>	CCNA1	NM_003914	1·13E-05	Cyclin A1
AIFM2NML0327972-30E-05Apoptosis-inducing factor, mitochondron-associated, 2PRKDCNML0060042-68E-05Protein kinase, DNA-activated, catalytic polypeptideSIKT7NML016532-88E-05Homo sapiens sirtuin (silent mating type information regulation 2 homologue) 7AKT3NML018004-22E-05v-akt matine thymoma viral oncogene homologue 3 (protein kinase, B, gamma)SILANML020348-63E-05Tuberous sclerosis iral oncogene homologue 4 (avian)TSC1NML020348-63E-05Chromosome 20 open reading frame 74C20orf74NML0023920-000127075Beta-2-microglobulinMDM2NML0023920-000127075Beta-2-microglobulinMST4NML0103300-000234023MST4 fishinding protein homologue (monecytic leukaemia) 4GADD45BNML016300-000278452Protein kinase, AMP-activated, gamma 2 non-calalytic subunitRB1NML002190-00034025Retinoblastoma 1 (including oscensoroma)PIKXCBNML008750-000839083Mitogen-activated protein kinase, 13IGF1RNML008750-000839082Insulin-like growth factor 1 receptorHK2NML001800-0013122NIC12 binding component 3GGRUA1NML0025920-0011742Proliferating cell nacker antigenBKC3(PUMA)AF54640-0013123GT2se activating Rap/RanGAP domain-like 1NFATC21PNML002390-00126077BC12 binding component 3GARNL1NML01530-00126077Dizze associated factor 2PCNANML002390-00126077 <td>FASLG</td> <td>NM_000639</td> <td>1·40E-05</td> <td>Fas ligand (TNF superfamily, member 6)</td>	FASLG	NM_000639	1·40E-05	Fas ligand (TNF superfamily, member 6)
PKRDCNM_0069042.68E-05Protein kinase, DNA-activated, catalytic polyeptideSIR17NM_0165382.83E-05Homo sapiers sirtuin (silent mating type information regulation 2 homologue) 7AKT3NM_118004.22E-05v-rel reticuloendotheliosis viral oncogene homologue 3 (protein kinase B, gamma)RELANM_0201366.25E-05Tuberous sciencosis 1C20orf74NM_000480.00012707Beta-2-microglobulinMDM2NM_000480.00012707Beta-2-microglobulinMDM2NM_0012300.00012374MDM2 p.55 hinding protein homologue (mouse)MYST4NM_012300.00023443Growth arrest and DNA-damage-inducible, betaRRAG2NM_0166010.00027452Protein kinase, AMP-activated, gamma 2 non-catalytic subunitRB1NM_0005210.00034063Retinoblastoma 1 (including osteosarcoma)PKXCBNM_0008750.00086087Insulin-ikie growth factor 1 receptorMAPK13NM_0027540.000908611Hecokinase 2TRAF2NM_001311TNF receptor-associated factor 2PCNANM_0013200.001174Protein kinase, AMP-activated, gamma 1 non-catalytic subunitRAF2NM_0013113GTPasa cativating Rap/RanGAP domain-like 1RAF4NM_0014900.0011323GTPasa cativating Rap/RanGAP domain-like 1RAF4NM_0012900.0011320GTPasa cativating Rap/RanGAP domain-like 1RAF4NM_0012900.0011322Nuclear factor dactivated T-cells, cytoplasmic, calcinPCNANM_0012900.0012570Protein kinase, AMP-	AIFM2	NM_032797	2·30E-05	Apoptosis-inducing factor, mitochondrion-associated, 2
SIRT7NM_0163382.88E-05Home sapiene situin (silent mating type information regulation 2 homologue) 7AKT3NM_1816904.22E-05v-akt murine thymoma viral oncogene homologue 3 (protein kinase B, gamma)RELANM_0015754.99E-05v-el reticuloubleiosis viral oncogene homologue 3 (protein kinase B, gamma)TSC1NM_0003686.25E-05Tuberous sclerosis 1C20erf74NM_0023438.63E-05Chromosome 20 open reading frame 74B2MNM_0023920.00012705Beta-2-microglobulinMDM2NM_0023200.00023403MDM2 p35 binding protein homologue (mouse)MYS14NM_0156750.00023463Growth arrest and DNA-damage-inducible, betaPKKAC2NM_0156750.00023463Growth arrest and DNA-damage-inducible, betaPKKAC3NM_0005100.00057466Phosphoinositide-3-kinase, catalytic, beta polypeptideMPK13NM_0005740.00058988Mitogen-activated protein kinase 13IGFIRNM_000750.000808611Hecokinase 2RKAC2NM_0007500.000809861Hecokinase 2PCNANM_0025920.0011291TNF receptor-associated factor 2PCNANM_0025920.0011291TNF receptor-associated factor 2PCNANM_0025920.00113132Grapase A, and protein banding, calcinPCAFNM_0012840.0012607BCL2 binding component 3GARNL1NM_0025920.00113122Nuclear factor of activated fractor 2PCNANM_0025920.0012607BCL2 binding cortin 2, 48 kDaSI	PRKDC	NM_006904	2.68E-05	Protein kinase, DNA-activated, catalytic polypeptide
AKT3NM_1816904.22E-05v-akt murine thymoma viral oncogene homologue 3 (protein kinase B, gamma)RELANM_0219754.99E-05v-rel reticuloendotheliosis viral oncogene homologue A (avian)TSC1NM_0003686.25E-05Tuberous sciencis 1C200r74NM_0023438.63E-05Chromosome 20 open reading frame 74B2MNM_0023920.000127075Beta-2-microglobulinMDM2NM_0023920.000127075Beta-2-microglobulinMDM2NM_0123300.000274101MYST histone acetyfransferase (monocytic leukaemia) 4GADD458NM_0156750.000254450Growth arrest and DNA-damage-inducible, betaPRKAG2NM_0162030.000274452Protein kinase, AMP-activated, gamma 2 non-catalytic subunitRB1NM_000310.00037465Phosphoniositide-3-kinase, catalytic, beta polypeptideMAPK13NM_0027540.000398988Mitogen-activated protein kinase 13IGFIRNM_0001890.00039861Hexokinase 2TRAF2NM_001890.0011940TNF receptor-associated factor 2PCNANM_0023150.00112057BGL2 binding component 3GARN11NM_0149900.00131223Crase activating Rap/RanGAP domain-like 1NFATC2IPNM_038450.0015207protein kinase 2PCAFNM_001780.00232031Catapase 9, apotosis-related cyteine peptidaseCAF2NM_001780.00232031Catapase 9, apotosis-related cyteine peptidaseCAR50NM_001750.0042805Sertin 2PRXAG1NM_01735	SIRT7	NM_016538	2·83E-05	Homo sapiens sirtuin (silent mating type information regulation 2 homologue) 7
RELA NM_021975 4.99E-05 v-rel reticuloendotheliosis viral oncogene homologue A (avian) TSC1 NM_000368 6-25E-05 Tuberous sclerosis 1 C20of74 NM_020343 8-63E-05 Chromosome 20 open reading frame 74 B2M NM_002392 0-000162073 Beta-2-microglobulin MDM2 NM_002392 0-000243101 MYST fistone acetyltransferase (monocytic leukaemia) 4 GADD45B NM_015675 0-000234032 Retinoblastoma 1 (including osteosarcoma) PRKAG2 NM_0106219 0-00034025 Retinoblastoma 1 (including osteosarcoma) PIKASCB NM_000211 0-00034025 Retinoblastoma 1 (including osteosarcoma) PIKASCB NM_000321 0-00034025 Retinoblastoma 1 (including osteosarcoma) PIKASCB NM_000875 0-000808872 Insulin-like growth factor 1 receptor RKAP NM_00189 0-00012911 TNF receptor-associated factor 2 PCNA NM_002592 0-0011794 Proliferating call mander antigen BBC3(PUMA) AF354654 0-00131322 Nuclar factor of activated T-cells, cytoplasmic, calcin PCAF	AKT3	NM_181690	4·22E-05	v-akt murine thymoma viral oncogene homologue 3 (protein kinase B, gamma)
TSC1 NM_000368 6 25E-05 Tuberous sclerosis 1 C200774 NM_020343 8 63E-05 Chromosome 20 open reading frame 74 B2M NM_002392 0.000127075 MDMZ p53 binding protein homologue (nouse) MDM2 NM_012330 0.000243101 MYST histone acetyltransferase (monocytic leukaemia) 4 GADD45B NM_016203 0.00023482 Protein kinase, AMP-activated, gamma 2 non-catalytic subunit RB1 NM_006219 0.00034025 Retinoblastoma 1 (including osteosarcoma) PIKAG2 NM_006219 0.000394025 Retinoblastoma 1 (including osteosarcoma) PIKAG3 NM_000774 0.000800872 Insulin-like growth factor 1 receptor HK2 NM_00189 0.000800871 Insulin-like growth factor 1 receptor HK2 NM_00189 0.00120911 TNF receptor-associated factor 2 PCNA NM_002510 0.00131323 GTPasa cativated, gamma 1 non-catalytic subunit RAFA12 NM_001890 0.00131323 GTPasa cativated, gamma 1 non-catalytic subunit RAFA12 NM_002810 0.00131323 GTPasa GTPasa AMACO140 AM	RELA	NM 021975	4·99E-05	v-rel reticuloendotheliosis viral oncogene homologue A (avian)
C20orf74 NM_0020343 8-63E-05 Chromosome 20 open reading frame 74 BZM NM_004048 0000127075 Beta-2-microglobulin MDM2 NM_002392 0000023101 MDYT Sibinding protein homologue (mouse) MYST4 NM_012330 000023403 Growth arrest and DNA-damage inducible, beta PKKAC2 NM_016030 0000334025 Retinoblastoma 1 (including ostosarcoma) PKKAC3 NM_000210 000034025 Retinoblastoma 1 (including ostosarcoma) PKKAC3 NM_000219 000034966 Phosphoinositide-3-kinase, catalytic, beta polypeptide MAPK13 NM_0002754 00009898 Mitogen-activated protein kinase 13 ICFIR NM_000189 000008072 Insulin-like growth factor 1 receptor HK2 NM_00178 000102911 TNF receptor-asociated factor 2 PCNA NM_002592 00011794 Proliferating cell nuclear antigen BGC3(PUMA) AF54654 00013132 GTPase activating Rap/RanGAP domain-like 1 NFATC2IP NM_00129 00015707 protein kinase 2 PCAF NM_00129 00015207	TSC1	NM 000368	6·25E-05	Tuberous sclerosis 1
B2MNM_004480.000127075Beta-2-microglobulinMDM2NM_0023920.000162073MDM2 p53 binding protein homologue (mouse)MTST4NM_0123300.000231463Growth arrest and DNA-damage-inducible, betaGADD45BNM_0167050.000234162Protein kinase, AMP-activated, gamma 2 non-catalytic subunitRBINM_0002110.00037452Protein kinase, AMP-activated, gamma 2 non-catalytic subunitPIKAG2NM_0062190.00037452Protein kinase, catalytic, beta polypeptideMAPK13NM_00027540.000598988Mitogen-activated protein kinase 13IGF1RNM_0007550.000908611Hexokinase 2TRAF2NM_001890.000908611Hexokinase 2TRAF2NM_001890.00129011TNF receptor-asociated factor 2PCNANM_0025920.0011749Proliferating cell nuclear antigenBBC3(PUMA)AF3546540.00126057BCL2 binding component 3GARNL1NM_0128950.0014721p300/CBP-asociated factorPKAG1NM_012890.0014721p300/CBP-asociated factorPKAG2NM_001290.0013520Cryben-asociated factorCASP9NM_001290.00134231Capase A, oppotosi-related cysteine peptidaseCDK2NM_001290.00232801Cyclin-dependent kinase 2DDB2NM_001790.00252077Damage-specific DNA-binding protein 2, 48 kDaSIR76NM_016390.00335452BCL2-asociated X proteinSISN2NM_0014500.00335452Settria 2PPP2CAN	C20orf74	NM 020343	8.63E-05	Chromosome 20 open reading frame 74
MDM2NA_0023920000162073MDM2 p53 binding protein homologue (mouse)MYST4NM_01623000002343101MYST histone acetyltransferase (monocytic leukaemia) 4GADD458NM_0156750000235452Protein kinase, AMP-activated, gamma 2 non-catalytic subunitRBINM_0002110000334025Retinoblastoma 1 (including ostoosarcoma)PIK3CBNM_000219000054966Phosphoinositide-3-kinase, catalytic, beta polypeptideMAPK13NM_0002754000058988Mitogen-activated protein kinase 13ICF1RNM_000754000098988Mitogen-activated protein kinase 13ICF1RNM_000189000098011Hexokinase 2PCNANM_00259200011794Proliferating cell nuclear antigenBBC3(PUMA)AF35465400012057BCL2 binding component 3GARNL1NM_0032815000131322Suclear factor of activated 1-cells, cytoplasmic, calcinPCAFNM_00384400014711p300/CBP-associated factorPKAGGNM_0122900194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_00179000258017Damase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_00179000258017Damase, AMP-activated, gamma 2 nonologue) 6 (S. cerevisiae)SIRT6NM_01633900026806Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_00175000395825Settri 2PPP2CANM_00151000395825Settri 2PPP2CANM_00151000395825Settri 2PPP	B2M	NM 004048	0.000127075	Beta-2-microglobulin
MYST4NM_0123300.000243101MYST histone acetyltransferase (monocytic leukaemia) 4GADD45BNM_0156750.00023463Growth arrest and DNA-damage-inducible, betaPRKAG2NM_0162030.00027842Protein kinase, AMP-activated, gamma 2 non-catalytic subunitRB1NM_000210.00034025Retinoblastoma 1 (including osteosarcoma)PIKXGBNM_00027540.000598988Mitogen-activated protein kinase 13IGF1RNM_0008750.000598988Mitogen-activated protein kinase 13IGF1RNM_0008750.000908611Hexokinase 2TRAF2NM_0011380.0012911TNF receptor-associated factor 2PCNANM_0025920.0011794Proliferating cell nuclear antigenBCG70UMA)AF3546540.00126057BCL2 binding component 3GARNL1NM_0149900.00131323GTPase activated T-cells, cytoplasmic, calcinPCAFNM_0028440.0014721p300/CBP-associated factorPCAFNM_0012290.00132301Cyclin-dependent kinase, 4MP-activated, gamma 1 non-catalytic subunitCASP9NM_0012290.00132801Cyclin-dependent kinase 2DB2NM_0016590.00232001Cyclin-dependent kinase 2DB2NM_001700.00232001Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0016540.0033452BCL2-associated factorSIRT6NM_001310.0033452BCL2-associated X proteinSIRT6NM_001320.0033452BCL2-associated X proteinSIRT6NM_001310.0033452 <t< td=""><td>MDM2</td><td>NM 002392</td><td>0.000162073</td><td>MDM2 p53 binding protein homologue (mouse)</td></t<>	MDM2	NM 002392	0.000162073	MDM2 p53 binding protein homologue (mouse)
GADD45BNM_0156750.000253463Growth arrest and DNA-damage-inducible, betaPRKAG2NM_0162030.000278452Protein kinase, AMP-activated, gamma 2 non-catalytic subunitRB1NM_0003210.00034025Retinoblastoma 1 (including osteosarcoma)PIK3CBNM_0062190.00057966Phosphoinositide 3-kinase, catalytic, beta polypeptideMAPK13NM_0027540.000598988Mitogen-activated protein kinase 13IGF1RNM_0001890.0009890Insulin-like growth factor 1 receptorHK2NM_0001890.00098611Hexokinase 2TRAF2NM_001890.00129111TNF receptor-associated factor 2PCNANM_0025920.0011794Proliferating cell nuclear antigenBG3(PUMA)AF3546540.00126057BC12 binding component 3GARNL1NM_0149900.00131323GTPase activating Rap/RanGAP domain-like 1NFATC2IPNM_0328150.00131522Nuclear factor of activated T-cells, cytoplasmic, calcinPCAFNM_0018840.0014721p300/CBP-associated factorPKAG1NM_2124610.00155707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_001790.00232801Cyclin-dependent kinase 2DDB2NM_001790.0035452Birtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_004340.0035452Birtuin (silent mating type information regulation 1, 91 kDaSIST1NM_001350.00349855Sestrin 2PPP2CANM_001710.0032907Immunoglob	MYST4	NM 012330	0.000243101	MYST histone acetyltransferase (monocytic leukaemia) 4
PKKAC2NM_0162030-000278452Protein kinase, AMP-activated, gamma 2 non-catalytic subunitRB1NM_0002110-00034025Retinoblastoma 1 (including soctosarcoma)PIK3CBNM_0002190-000547966Phosphoinositide-3-kinase, catalytic, beta polypeptideMAPK13NM_0027540-00059898Mitogen-activated protein kinase 13IGF1RNM_0008750-000080872Insulin-like growth factor 1 receptorHK2NM_0011890-000908611Hexokinase 2TRAF2NM_0025920-001794Proliferating cell nuclear antigenBBC3(PUMA)AF3546540-0012057BCL2 binding component 3GARNL1NM_0149900-00131223GTPase activating Rap/RanGAP domain-like 1NFATC21PNM_0232150-00131522Nuclear factor of activated T-cells, cytoplasmic, calcinPCAFNM_0012890-0019771p300/CBP-associated factorPKAGGINM_2124610-0015577protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_001290-001232801Cyclin-dependent kinase 2DDB2NM_001790-00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_003140-0035452BCL2-associated X proteinSKN2NM_001530-0035452Scl2-associated X proteinStAT1NM_0073150-0035452Scl2-associated X proteinStAT1NM_0015100-0035925Sestrin 2PPP2CANM_001510-0035927Immunoglobulin (CD79A) binding protein 1, 91 kDaSESN2NM_001510-0035927	GADD45B	NM_015675	0.000253463	Growth arrest and DNA-damage-inducible, beta
RBINM_0003210-000334025Retinoblastoma 1 (including osteosarcoma)PIKACBNM_0062190-000547966Phosphoinositide-3-kinase, catalytic, beta polypeptideMAPK13NM_0027540-000598988Mitogen-activated protein kinase 13IGF1RNM_0008750-000800872Insulin-like growth factor 1 receptorHK2NM_001890-000908611Hexokinase 2TRAF2NM_0025920-0011794Proliferating cell nuclear antigenBBC3(PUMA)AF3546540-00126057BCL2 binding component 3GARNL1NM_0028920-0011794Proliferating cell nuclear antigenPCAFNM_003840-0014721p300/CBP-associated factorPCAFNM_003840-0014721p300/CBP-associated factorPCAFNM_003840-0014721p300/CBP-associated factorPKAGG1NM_2124610-0015707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_001790-00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0013150-0033452BCL2-associated X proteinSTAT1NM_003150-0035452Sestrin 2PPP2CANM_001510-0044978Signal transducer and activator of transcription 1, 91 kDaSISN2NM_001510-004392207Imaserition factor, RNA polymerase ISIAH1NM_00056100-0035207Imaserition factor, RNA polymerase ISISN2NM_001510-0035452Sestrin 2PP2CANM_001510-0035452Sestrin 2SIAT1NM_00151	PRKAG2	NM_016203	0.000278452	Protein kinase. AMP-activated, gamma 2 non-catalytic subunit
PIX3CBNM_00027190.000547966Phosphoinositide-3-kinase, catalytic, beta polypeptideMAPK13NM_0027540.000589888Mitogen-activated protein kinase 13IGF1RNM_0001890.000908011Hexek coloredHK2NM_0001890.000908011Hexek coloredTRAF2NM_0211380.0012911TNF receptor-associated factor 2PCNANM_0025920.0011794Proliferating cell nuclear antigenBBC3(PUMA)AF3546540.00126057BCL2 binding component 3GARNL1NM_0149900.00131323GTPase activating Rap/RanGAP domain-like 1NFATC2IPNM_0328150.0013522Nuclear factor of activated T-cells, cytoplasmic, calcinPCAFNM_0038840.0014721p300/CBP-associated factorPRKAG1NM_2124610.0015707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_001290.00194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_001700.00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_00165390.0035452BCL2-associated X proteinSTAT1NM_0073150.0035452BCL2-associated X proteinSESN2NM_0012190.000395452BCL2-associated X proteinSIAH1NM_0000510.0035452BCL2-associated X proteinUBTFNM_0142330.0035452Sestrin 2PP2CANM_0005100.00552907Immunoglobulin (CD79A) binding protein 1ATMNM_0000510.005052907Immunoglobulin (CD79A) binding protein	RB1	NM_000321	0.000334025	Retinoblastoma 1 (including osteosarcoma)
MAPK13NM_0027540.000598988Mitogen-activated protein kinase 13IGF1RNM_0008750.00080872Insulin-like growth factor 1 receptorHK2NM_001890.000908611Hexokinase 2TRAF2NM_0021920.0011794Proliferating cell nuclear antigenBBC3(PUMA)AF3546540.00126057BCL2 binding component 3GARNL1NM_0123810.00131322Nuclear factor of activated T-cells, cytoplasmic, calcinPCAFNM_0038840.0014721p300/CBP-associated factorPRKAGINM_2124610.00155707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_0012290.0014233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_0017980.00232017Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_00165390.0035452BCL2-associated X proteinSESN2NM_00173150.0035452BCL2-associated X proteinSTAT1NM_0073150.0035452Sestrin 2PPP2CANM_00106100.0043647Votein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_014230.0035825Sestrin 2PPP2CANM_001510.00532907Immunoglobulin (CD79A) binding protein 1ATMNM_000510.00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_001510.00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_0049580.00782529Fas forbiding protein 1RHEBL1NM_0049580.007825916Ras homologue e	PIK3CB	NM_006219	0.000547966	Phosphoinositide-3-kinase, catalytic, beta polypeptide
InstitutionInstitutionIGFIRNM_0008750000800872Insulin-like growth factor 1 receptorHK2NM_001890000908611Hexokinase 2TRAF2NM_01178400102911TNF receptor-associated factor 2PCNANM_00259200011794Proliferating cell nuclear antigenBBC3(PUMA)AF354654000126057BCL2 binding component 3GARNL1NM_0149900-00131323GTPase activating Rap/RanGAP domain-like 1NFATC2IPNM_0388150-0014721p300/CBP-associated factorPCAFNM_0038840-0014721p300/CBP-associated factorPRKAG1NM_2124610-00155707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_0012290-00194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_001290-00232801Cyclin-dependent kinase 2DDB2NM_0015390-00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (<i>S. cerevisiae</i>)BAXNM_0043140-0035452BCL2-associated X proteinSTAT1NM_0073150-0044978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_001070-00252077Damage-specific Dival-pition factor, RNA polymerase ISIAH1NM_0005160-00395455Sertin 2PPP2CANM_0017150-0044361Protein phosphatase 2 (formerhy 2A), catalytic subunit, alpha isoformUBTFNM_0166100-0059207Imastinia finding transcription factor, RNA polymerase ISIAH1NM_001066100	MAPK13	NM_002754	0.000598988	Mitogen-activated protein kinase 13
HK2NM_0001890000908611Hexokinase 2TRAF2NM_021138000102911TNF receptor-associated factor 2PCNANM_00259200011794Proliferating cell nuclear antigenBBC3(PUMA)AF354654000126057BCL2 binding component 3GARNLINM_014990000131323GTPase activating Rap/RanGAP domain-like 1NFATC2IPNM_032815000131522Nuclear factor of activated T-cells, cytoplasmic, calcinPCAFNM_0038840.0014721p300/CBP-associated factorPRKAGINM_011290.0012320Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_001780.00232801Cyclin-dependent kinase 2DDB2NM_001070.00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0043240.00335452BCL2-associated X proteinSTAT1NM_0073150.0044978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_011590.004361Protein phosphatase 2 (Forrerly 2A), catalytic subunit, alpha isoformUBFNM_000510.0050669Ataxia telangiectasia mutatedIGBP1NM_0015510.00532907Immunoglobulin (CD79A) binding protein 1ATMNM_003840.0052206FK506 binding protein 1ATMNM_000510.0052906Ataxia telangiectasia mutatedIGBP1NM_0015510.0053492Sestrin 2PP2CANM_001510.0052907Immunoglobulin (CD79A) binding protein 1ATMNM_000510.0052206FK506 binding transcription factor	IGF1R	NM_000875	0.000800872	Insulin-like growth factor 1 receptor
IntegrationOrder StateTRAF2NM_0011380.00102911TNF receptor-associated factor 2PCNANM_0025920.0011794Proliferating cell nuclear antigenBBC3(PUMA)AF3546540.00126057BCL2 binding component 3GARNL1NM_0149900.00131323GTPase activating Rap/RanGAP domain-like 1NFATC2IPNM_0328150.00131522Nuclear factor of activated T-cells, cytoplasmic, calcinPCAFNM_0038840.0014721p300/CBP-associated factorPRKAG1NM_2124610.00155707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_0012290.00194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_0017980.00232801Cyclin-dependent kinase 2DDB2NM_001070.00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0163390.00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (<i>S. cerevisiae</i>)BAXNM_0043240.00395825Sestrin 2PP2CANM_0027150.0044978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_014530.0035845Upstream binding transcription factor, RNA polymerase 1SIAH1NM_00066100.00479628Seven in absentia homologue 1 (Drosophila)ATTNM_0000510.00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_1445930.00552916Ras homologue enriched in brain like 1FADDNM_0038440.00782508FK506 binding protein 12-rapamycin associated	HK2	NM_000189	0.000908611	Hexokinase 2
NM_00NM_000OON1794Proliferating cell nuclear antigenBBC3(PUMA)AF3546540-00126057BCL2 binding component 3GARNL1NM_0149900-00131323GTPase activating Rap/RanGAP domain-like 1NFATC2IPNM_0328150-00131522Nuclear factor of activated T-cells, cytoplasmic, calcinPCAFNM_003840-0014721p300/CBP-associated factorPRKAG1NM_2124610-00155707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_0012290-00194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_0017980-00232801Cyclin-dependent kinase 2DB2NM_001070-00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0165390-00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_0043240-0035452BCL2-associated X proteinSTAT1NM_0073150-00344978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0314590-0035825Sestrin 2PPP2CANM_0027150-004043189Upstream binding transcription factor, RNA polymerase ISIAH1NM_00106100-00479628Seven in absentia homologue 1 (Drosophila)ATMNM_0005110-00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_1445930-00552916Ras homologue enriched in brain like 1FADDNM_0035460-00782508FK506 binding protein 12-rapamycin associated protein 1TD53NM_0026400-	TRAF2	NM 021138	0.00102911	TNF receptor-associated factor 2
RestRestRestBGC3(PUMA)AF364540-00126057BCL2 binding component 3GARNL1NM_0149900-00131323GTPase activating Rap/RanGAP domain-like 1NFATC2IPNM_0328150-0013522Nuclear factor of activated T-cells, cytoplasmic, calcinPCAFNM_0038840-0014721p300/CBP-associated factorPRKAGINM_2124610-00155707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_0012290-00194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_001780-00232001Cyclin-dependent kinase 2DDB2NM_001070-00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0165390-00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_0043240-00335452BCL2-associated X proteinSTAT1NM_0073150-0044978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0142330-00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_000510-0049628Seven in absentia homologue 1 (Drosophila)ATMNM_000510-00532907Immunoglobulin (CD79A) binding protein 1RHEB1NM_0049580-00532907Immunoglobulin (CD79A) binding protein 1FADDNM_004540-00532907Immunoglobulin (CD79A) binding protein 1FADDNM_0026400-0054258FK506 binding protein 12-rapamycin associated protein 1TFS3NM_0026400-0084807Phosphoino	PCNA	NM_002592	0.0011794	Proliferating cell nuclear antigen
Base of the first of the fir	BBC3(PUMA)	AF354654	0.00126057	BCL2 binding component 3
NFATC2IPNM_0328150-0013152Nuclear factor of activated T-cells, cytoplasmic, calcinPCAFNM_0038840-0014721p300/CBP-associated factorPRKAGINM_2124610-00155707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_0012290-00194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_0017980-00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0165390-00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (<i>S. cerevisiae</i>)BAXNM_0043240-00335452BCL2-associated X proteinSTAT1NM_0073150-0044978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0142330-0044961Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBFNM_0005110-0044961Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBFNM_001066100-00479628Seven in absentia homologue 1 (Drosophila)ATMNM_0005510-0050669Ataxia telangiectasia mutatedIGBP1NM_0015510-0052916Ras homologue enriched in brain like 1FADDNM_0038240-00522808FK506 binding protein 12-rapamycin associated protein 1TP53NM_0005460-00844817Tumour protein p53 (Li-Fraumeni syndrome)PIKAGGNM_0026490-00844817Phosphoinositie-3-kinase, catalytic, gamma polypeptidePM1DBC0424180-0095229Protein phosphatase 1D magnesium-dependent, delta isoform	GARNL1	NM 014990	0.00131323	GTPase activating Rap/RanGAP domain-like 1
PCAFNM_0038840.0014721p300/CBP-associated factorPCAFNM_0012290.00155707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_0012290.00194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_0017980.00232801Cyclin-dependent kinase 2DDB2NM_0016390.00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_0043240.00335452BCL2-associated X proteinSTAT1NM_0073150.0034978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0142390.00431895Sestrin 2PPP2CANM_0027150.00404361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_01026100.00479628Seven in absentia homologue 1 (Drosphila)ATMNM_0000510.00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_0038240.0052916Ras homologue enriched in brain like 1FADDNM_003840.00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0026400.00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026410.00840817Phosphatase, catalytic, gamma polypeptidePPM1DBC0424180.0055229Protein phosphatase 1D magnesinu-dependent, delta isoform	NFATC2IP	NM 032815	0.00131522	Nuclear factor of activated T-cells, cytoplasmic, calcin
PRKAGINM_2124610-00155707protein kinasePRKAGINM_2124610-00155707protein kinase, AMP-activated, gamma 1 non-catalytic subunitCASP9NM_0012290-00194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_001070-00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0165390-00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_0043240-00335452BCL2-associated X proteinSTAT1NM_0073150-00344978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0014590-00395825Sestrin 2PPP2CANM_0027150-00404361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_0142330-00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_000510-0050669Ataxia telangiectasia mutatedIGBP1NM_0015510-00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_0038240-00551322Fas (TNFRSF6)-associated via death domainFRAP1NM_0049580-00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0026490-00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePM1DBC0424180-0052229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140-0104375Ras homologue enriched in brain	PCAF	NM_003884	0.0014721	p300/CBP-associated factor
AndreadAndreadBrother and sectorCASP9NM_001290-00194233Caspase 9, apoptosis-related cysteine peptidaseCDK2NM_0017980-00232801Cyclin-dependent kinase 2DDB2NM_001070-00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0165390-00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_0043240-00335452BCL2-associated X proteinSTAT1NM_0073150-00344978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0142330-004361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_0142330-00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_0000510-00500669Ataxia telangiectasia mutatedIGBP1NM_001510-00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_0038240-0051322Fas (TNFRSF6)-associated via death domainFAAP1NM_0049580-00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0026490-00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180-0095229Protein phosphatase 10RHEBNM_0056140-0014275Ras homologue enriched in brain	PRKAG1	NM 212461	0.00155707	protein kinase. AMP-activated, gamma 1 non-catalytic subunit
CDK2NM_0017980.00232801Cyclin-dependent kinase 2DDB2NM_001070.00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0165390.00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_0043240.00335452BCL2-associated X proteinSTAT1NM_0073150.00344978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0314590.00395825Sestrin 2PPP2CANM_0027150.0044361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_010266100.00479628Seven in absentia homologue 1 (Drosophila)ATMNM_000510.0050669Ataxia telangiectasia mutatedIGBP1NM_001510.00552916Ras homologue enriched in brain like 1FADDNM_0038240.00552916Ras homologue enriched in brain like 1FADDNM_0049580.00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0026490.00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPMIDBC0424180.0095229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140.0104375Ras homologue enriched in brain	CASP9	NM 001229	0.00194233	Caspase 9. apoptosis-related cysteine peptidase
DDB2NM_0001070-00252077Damage-specific DNA-binding protein 2, 48 kDaSIRT6NM_0165390-00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_0043240-00335452BCL2-associated X proteinSTAT1NM_0073150-00344978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_014590-00395825Sestrin 2PPP2CANM_0027150-00404361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_0142330-00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_001066100-00479628Seven in absentia homologue 1 (Drosophila)ATMNM_0000510-00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_0145330-00552916Ras homologue enriched in brain like 1FADDNM_0038240-00561322Fas (TNFRSF6)-associated via death domainFRAP1NM_0005460-00814257Tumour protein positide-3-kinase, catalytic, gamma polypeptidePM1DBC0424180-00952299Protein phosphatase 1D magnesium-dependent, delta isoform	CDK2	NM 001798	0.00232801	Cyclin-dependent kinase 2
SIRT6NM_0165390.00268096Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)BAXNM_0043240.00335452BCL2-associated X proteinSTAT1NM_0073150.00344978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0314590.00395825Sestrin 2PPP2CANM_0027150.0044361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_0142330.00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_0000510.0050669Ataxia telangiectasia mutatedIGBP1NM_0015510.00552916Ras homologue enriched in brain like 1FADDNM_0038240.0051322Fas (TNFRSF6)-associated via death domainFRAP1NM_0005460.00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490.00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180.00952229Protein phosphatase 1D magnesium-dependent, delta isoform	DDB2	NM 000107	0.00252077	Damage-specific DNA-binding protein 2, 48 kDa
BAXNM_0043240-00335452BCL2-associated X proteinSTAT1NM_0073150-00344978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0314590-00395825Sestrin 2PPP2CANM_0027150-00404361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_0142330-00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_0010066100-00479628Seven in absentia homologue 1 (Drosophila)ATMNM_0000510-00500669Ataxia telangiectasia mutatedIGBP1NM_0115510-00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_0038240-00561322Fas (TNFRSF6)-associated via death domainFRAP1NM_0049580-00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0005460-00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490-00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180-00952229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140-0104375Ras homologue enriched in brain	SIRT6	NM 016539	0.00268096	Sirtuin (silent mating type information regulation 2 homologue) 6 (S. cerevisiae)
STAT1NM_0073150-00344978Signal transducer and activator of transcription 1, 91 kDaSESN2NM_0314590-00395825Sestrin 2PPP2CANM_0027150-00404361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_0142330-00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_00006100-00479628Seven in absentia homologue 1 (Drosophila)ATMNM_0000510-00500669Ataxia telangiectasia mutatedIGBP1NM_0115510-00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_0038240-00561322Fas (TNFRSF6)-associated via death domainFRAP1NM_0005460-00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490-00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180-0104375Ras homologue enriched in brain	BAX	NM 004324	0.00335452	BCL2-associated X protein
SESN2NM_0314590·00395825Sestrin 2PPP2CANM_0027150·00404361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_0142330·00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_0010066100·00479628Seven in absentia homologue 1 (Drosophila)ATMNM_0000510·00500669Ataxia telangiectasia mutatedIGBP1NM_0015510·00522907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_1445930·00561322Fas (TNFRSF6)-associated via death domainFADDNM_0038240·00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0005460·00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490·00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180·0052229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140·0104375Ras homologue enriched in brain	STAT1	NM_007315	0.00344978	Signal transducer and activator of transcription 1, 91 kDa
PPP2CANM_0027150·00404361Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoformUBTFNM_0142330·00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_0010066100·00479628Seven in absentia homologue 1 (Drosophila)ATMNM_0000510·00500669Ataxia telangiectasia mutatedIGBP1NM_0015510·00522907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_1445930·00552916Ras homologue enriched in brain like 1FADDNM_0038240·00561322Fas (TNFRSF6)-associated via death domainFRAP1NM_0005460·00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490·00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180·0052229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140·0104375Ras homologue enriched in brain	SESN2	NM 031459	0.00395825	Sestrin 2
UBTFNM_0142330·00431895Upstream binding transcription factor, RNA polymerase ISIAH1NM_0010066100·00479628Seven in absentia homologue 1 (Drosophila)ATMNM_0000510·00500669Ataxia telangiectasia mutatedIGBP1NM_0015510·00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_1445930·00561322Fas (TNFRSF6)-associated via death domainFADDNM_0038240·00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0005460·00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490·00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180·0052229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140·0104375Ras homologue enriched in brain	PPP2CA	NM 002715	0.00404361	Protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform
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IGBP1NM_0015510·00532907Immunoglobulin (CD79A) binding protein 1RHEBL1NM_1445930·00552916Ras homologue enriched in brain like 1FADDNM_0038240·00561322Fas (TNFRSF6)-associated via death domainFRAP1NM_0049580·00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0005460·00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490·00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180·00952229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140·0104375Ras homologue enriched in brain	ATM	NM 000051	0.00500669	Ataxia telangiectasia mutated
RHEBL1NM_1445930·00552916Ras homologue enriched in brain like 1FADDNM_0038240·00561322Fas (TNFRSF6)-associated via death domainFRAP1NM_0049580·00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0005460·00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490·00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180·00952229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140·0104375Ras homologue enriched in brain	IGBP1	NM_001551	0.00532907	Immunoglobulin (CD79A) binding protein 1
FADDNM_0038240·00561322Fas (TNFRSF6)-associated via death domainFRAP1NM_0049580·00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0005460·00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490·00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180·00952229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140·0104375Ras homologue enriched in brain	RHEBL1	NM 144593	0.00552916	Ras homologue enriched in brain like 1
FRAP1NM_0049580·00782508FK506 binding protein 12-rapamycin associated protein 1TP53NM_0005460·00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490·00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180·00952229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140·0104375Ras homologue enriched in brain	FADD	NM 003824	0.00561322	Fas (TNFRSF6)-associated via death domain
TP53NM_0005460·00814257Tumour protein p53 (Li-Fraumeni syndrome)PIK3CGNM_0026490·00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180·00952229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140·0104375Ras homologue enriched in brain	FRAP1	NM 004958	0.00782508	FK506 binding protein 12-rapamycin associated protein 1
PIK3CGNM_0026490·00840817Phosphoinositide-3-kinase, catalytic, gamma polypeptidePPM1DBC0424180·00952229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140·0104375Ras homologue enriched in brain	TP53	NM 000546	0.00814257	Tumour protein p53 (Li-Fraumeni syndrome)
PPM1DBC0424180.00952229Protein phosphatase 1D magnesium-dependent, delta isoformRHEBNM_0056140.0104375Ras homologue enriched in brain	PIK3CG	NM 002649	0.00840817	Phosphoinositide-3-kinase, catalytic, gamma polypeptide
RHEB NM_005614 0.0104375 Ras homologue enriched in brain	PPM1D	BC042418	0.00952229	Protein phosphatase 1D magnesium-dependent, delta isoform
	RHEB	NM_005614	0.0104375	Ras homologue enriched in brain

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Table 2.	(Continued)
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Gene	GenBank accession number	P-value	Definition
DNMT1	NM_001379	0.0106164	DNA (cytosine-5-)-methyltransferase 1
RRAS	NM_006270	0.0108314	Related RAS viral (r-ras) oncogene homologue
SIRT2	NM_012237	0.0115687	Sirtuin (silent mating type information regulation 2 homologue) 2
GADD45G	NM_006705	0.013215	Growth arrest and DNA-damage-inducible, gamma
PPP2CB	NM_001009552	0.0150046	Protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform
ZMAT3	NM_022470	0.015343	Zinc finger, matrin type 3
YWHAB	NM_003404	0.0166223	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide
YWHAQ	NM_006826	0.0168976	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, theta polypeptide
TNF	NM_000594	0.0177639	Tumour necrosis factor (TNF superfamily, member 2)
RPKAB1	NM_006253	0.020311	Protein kinase, AMP-activated, beta 1 non-catalytic subunit
NF1	NM_001042492	0.0224542	Neurofibromin 1
MLH1	NM_000249	0.0237558	mutL homologue 1, colon cancer, nonpolyposis type 2 (E. coli)
TOX4	NM_014828	0.0242946	TOX high-mobility group box family member 4
MAPK14	NM_001315	0.0277024	Mitogen-activated protein kinase 14
ZMAT2	NM_144723	0.0279075	Zinc finger, matrin type 2
KRAS	NM_033360	0.0351255	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue
MTA2	NM_004739	0.0357651	Metastasis associated 1 family, member 2
BCL2A1	NM_004049	0.0384042	BCL2-related protein A1
RBL1	NM_002895	0.0424297	Retinoblastoma-like 1 (p107)
NFKB1	NM_003998	0.0430098	Nuclear factor of kappa light polypeptide gene enhancer

the amount of full-length p53 and a 1.98-fold increase in the amount of the beta/gamma isoform, which was significant at 48 hr (Fig. 2b). Induction of p21 and Bax proteins using western blot assays was performed for 12 individuals. Densitometric analysis of western blots showed a 3.8-fold increase in p21 (P < 0.001) and a 1.39-fold increase in Bax (P < 0.05) following 48 hr of culture with IFN- β (Fig. 2d,e). These studies support the hypothesis that IFN- β may activate the p53 signal pathway in PBMC which is critical for cell proliferation and apoptosis.

Differences in the induction of p53 and p53 isoforms following gamma irradiation (IR) and upon culture with IFN- β

As gamma IR is a potent inducer of p53, we compared the induction of p53 and its beta/gamma isoform following either treatment with gamma IR or the addition of 1000 IU/ml IFN- β for 48 hr. As shown in Fig. 3(a,c), gamma IR (10 Gy) of PBMC induced the expression principally of full-length p53. Treatment with IFN- β , in contrast, induced both the full-length and beta/gamma isoforms of p53. In three volunteers, the beta/gamma isoform was dominant over the full-length isoform (Fig. 3b,d). These studies suggest that the p53 activation patterns of IFN- β are different from those of genotoxic stress, the most well-known inducer of p53.

Induction of STAT1 and STAT2 by IFN- β

The IFN- β receptor uses the Jak-STAT pathway to transduce signals necessary for the transcription of IFNresponsive genes. Also, STAT1 and STAT2 form part of the heterotrimeric complex that binds to the promoter regions of p53. We examined whether STAT1 and STAT2 are targets for IFN- β , and thus act to amplify the IFN- β signalling pathway. We examined the expression of STAT1 and STAT2 following culture of PBMC with IFN- β from 12 healthy volunteers. As shown in Fig. 4(a-c), there was a significant increase in protein levels of both STAT1 and STAT2 as early as 24 hr after culture using western blotting techniques. Densitometric studies showed a twofold increase over baseline for the induction of STAT2, while STAT1 showed a 3.7-fold increase (P < 0.05 compared with untreated cells for both STAT1 and STAT2).

To determine whether the increased expression of STAT1 and STAT2 was attributable to an increase in the mRNA of the respective STAT1 and STAT2 genes, realtime RT-PCR using primers specific for STAT1 and STAT2 was performed on PBMC. In mRNA obtained from the PBMC of seven individuals cultured with IFN- β , real-time RT-PCR values showed a 5.6-fold increase in mRNA levels over baseline for STAT1 and a 5.4-fold increase for STAT2 at 48 hr (Fig. 4d,e). Kinetic studies showed that the increase in mRNA for both STAT1 and

Table 3.	Identification	of p53	response	pathway	genes	that	play a	role in	cell	cycle	arrest	and	apoptosis
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	GenBank accession	Regulation by		
Gene	number	interferon- β	P-value	Definition
Apoptosis				
PIK3CG	NM_002649	_	0.008408	Phosphoinositide-3-kinase, catalytic, gamma polypeptide
PIK3CB	NM_006219	_	0.000548	Phosphoinositide-3-kinase, catalytic, beta polypeptide
NFKB1	NM_003998	+	0.04301	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
FADD	NM_003824	+	0.005613	Fas (TNFRSF6)-associated via death domain
TRAF2	NM_021138	+	0.001029	TNF receptor-associated factor 2
TNF	NM_000594	+	0.017764	Tumour necrosis factor (TNF superfamily, member 2)
BCL2A1	NM_004049	+	0.038404	BCL2-related protein A1
CASP9	NM 001229	+	0.001942	Caspase 9, apoptosis-related cysteine peptidase
AIFM2	NM 032797	+	2·3E-05	Apoptosis-inducing factor, mitochondrion-associated, 2
MCL1	NM 021960	+	8.6E-09	Myeloid cell leukaemia sequence 1 (BCL2-related)
FASLG	NM 000639	+	1.4E-05	Fas ligand (TNF superfamily, member 6)
MDM2	NM 002392	+	0.000162	MDM2 p53 binding protein homologue (mouse)
FAS	NM 000043	+	6.3E-07	Fas (TNF receptor superfamily, member 6)
BBC3	AF354654	+	0.001261	BCL2 binding component 3
(PUMA)	111001001		0 001201	
PMAIP1	NM 021127	+	2.21E-06	Phorbol-12-myriSTATe-13-acetate-induced protein 1
(NOXA)	14141_021127	I	2 212 00	Thorbor 12 mynorrite 15 actuate induced protein 1
Cell cycle arrest				
PRKDC	NM 006904	_	2.68E-05	Protein kinase DNA-activated catalytic polypentide
C20orf74	NM_020343	_	2.00E.05	Chromosome 20 open reading frame 74
TSC2	NM_000548	_	1.12E-05	Tuberous sclerosis 2
TSC1	NM_000368	_	6.25E-05	Tuberous sciences 1
MVST4	NM_012330	_	0.000243	MVST histone acetultransferase (monocutic laukaemia) 4
CADNI 1	NM_014990		0.001313	GTPase activating Pan/PanCAP domain like 1
EDADI	NM 004058	_	0.007825	EVEQ6 binding protein 12 renewycin associated protein 1
FKAP1	NM_002805	-	0.007825	PK506 binding protein 12-rapaniychi associated protein 1
KBLI	NM_002895	-	0.04243	Retinoblastoma-like 1 (p107)
IWHAB	INM_003404	-	0.016622	stinution protoin hate nehrmantide
	ND (00/02/		0.01/000	activation protein, beta polypeptide
YWHAQ	NM_006826	+	0.016898	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase
DDI	ND (000221		0.000224	activation protein, theta polypeptide
KB1	NM_000321	+	0.000334	Retinoblastoma I (including osteosarcoma)
SESN2	NM_031459	+	0.003958	Sestrin 2
CDK2	NM_001798	+	0.002328	Cyclin-dependent kinase 2
PCNA	NM_002592	+	0.001179	Proliferating cell nuclear antigen
CCNA1	NM_003914	+	1·13E-05	Cyclin Al
CDKN1A	NM_078467	+	7·47E-07	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)
Overlapping				
IGF1R	NM_000875	-	3·39E-06	Insulin-like growth factor 1 receptor
AKT3	NM_181690	-	4·22E-05	v-akt murine thymoma viral oncogene homologue 3
				(protein kinase B, gamma)
IGF1R	NM_000875	-	0.000801	Insulin-like growth factor 1 receptor
ATM	NM_000051	-	0.005007	Ataxia telangiectasia mutated
GADD45G	NM_006705	-	0.013215	Growth arrest and DNA-damage-inducible, gamma
TP53	NM_000546	+	0.008143	Tumour protein p53 (Li-Fraumeni syndrome)
NFATC2IP	NM_032815	+	0.001315	Nuclear factor of activated T-cells, cytoplasmic, calcin
RELA	NM_021975	+	4·99E-05	v-rel reticuloendotheliosis viral oncogene homologue A
BAX	NM_004324	+	0.003355	BCL2-associated X protein
GADD45B	NM_015675	+	0.000253	Growth arrest and DNA-damage-inducible, beta

+, up-regulation; -, down-regulation.

STAT2 was seen early, at 6 hr (P < 0.05 compared with untreated cells). These studies show that IFN- β induces rapid transcription of STAT1 and STAT2, thereby increas-

ing the constitutive levels of the key signalling proteins necessary for the activation of the IFN- β receptor signalling pathway.



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Figure 1. Activation of the p53 signal pathway by interferon (IFN)- β in peripheral blood mononuclear cells (PBMC) at the transcription level. (a) Hierarchical cluster of 74 differentially regulated genes in the p53 signal pathway after culture of PBMC with IFN- β . Each row corresponds to a single gene and each column corresponds to the average relative expression level at each time-point, with 0, 24 and 48 hr from left to right. The values were transformed to a log₂ scale and converted into colour intensity. Red indicates increased expression and blue indicates reduced expression. (b–g) Real-time reverse transcription–polymerase chain reaction (RT-PCR) values for p53 and its target genes following culture with IFN- β : (b) p53, (c) Bcl-2-associated X protein (Bax), (d) NOXA, (e) PUMA, (f) p21 and (g) MDM2; pooled data from seven individuals. The *y*-axis represents the fold increase in real-time values after normalization to β -actin. **P < 0.001; *P < 0.05 when compared with unstimulated cells at 0 hr.

Induction of apoptosis in PBMC cultured with IFN- β

To examine the functional consequences of activation of p53, we investigated the apoptosis of PBMC following culture with IFN- β using flow cytometry. The addition of

IFN- β to PBMC and culture for 48 hr did not increase apoptosis when compared with cells cultured in medium alone. As Act D is a known inducer of apoptosis in a number of cell lines,³⁰ we examined the effects of addition of Act D to PBMC cultured with IFN- β . The addi-

Regulation of p53 by IFN- β



tion of IFN- β did not increase the number of Annexin V-stained cells. The percentage of Annexin V⁺ 7-AAD⁻ cells increased from 10.63 to 25.50% in the presence of

Act D. In the presence of both ActD and IFN- β , the percentage of Annexin V⁺ 7-AAD⁻ cells was 39·73% (P < 0.05; Fig. 5). These experiments showed that,

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Figure 3. Induction of p53 in peripheral blood mononuclear cells (PBMC) following gamma irradiation (IR) or after culture with interferon (IFN)- β . (a) Induction of p53 in PBMC from three individuals following culture with IFN- β (1000 IU/ml for 48 hr) and probing with anti-p53 antibody. (b) Densitometric analysis of p53 after normalization to β -actin. (c) Induction of p53 in PBMC from the same three individuals following gamma irradiation of PBMCs (10 Gy). (d) Densitometric analysis of p53 and its isomers, after normalization to β -actin. The *y*-axis in (b) and (d) represents the per cent increase in the signal of the full-length (FL) and beta/gamma (b/g) isoforms in cells subjected to either gamma irradiation or culture with IFN- β when compared with cells cultured in medium alone.



although there was an increase in the expression of proapoptotic genes following culture with IFN- β (Fig. 1), a direct effect of IFN- β on apoptosis was not evident unless a DNA-damaging agent was added.

IFN- β prevents exit from the G0/G1 stage of the cell cycle

Our microarray analysis, along with the real-time RT-PCR and western blot experiments, showed that the

Figure 4. Induction of signal transducers and activators of transcription 1 (STAT1) and STAT2 by interferon (IFN)- β . (a) Western blots of STAT1 and STAT2 proteins following culture of peripheral blood mononuclear cells (PBMC) with IFN- β from two individuals. (b) Densitometric values of protein levels for STAT1 and (c) densitometric values for STAT 2; pooled analysis for 12 individuals. (d, e) Results of real-time reverse transcription-polymerase chain reaction (RT-PCR) for (d) STAT1 and (e) STAT2 gene expression following culture of PBMC with IFN- β ; pooled analysis for seven individuals. Results are expressed as fold increase in mRNA levels over that seen following culture of PBMC in medium alone. *P < 0.05; **P < 0.001 when compared with cells at 0 hr.

expression of p21 was significantly elevated following culture with IFN- β . As p21 plays a critical role in inducing cell cycle arrest, we examined the effect of IFN- β on cell cycle progression. As the majority of fresh PBMC are non-proliferating (arrested in the G0/G1 stage), the cells were cultured with PHA to promote cell division in T cells, and the effect of the addition of IFN- β on cell cycle progression was examined using flow cytometry (Fig. 6). As expected, after the addition of PHA (10 µg/ml), the percentage of CD3⁺ lymphocytes in the G0/G1 stage in Figure 5. Flow cytometric analysis of induction of apoptosis by interferon (IFN)- β : (a) cells treated with medium alone, (b) cells treated with 1000 IU/ml IFN- β for 48 hr, (c) cells treated with actinomycin D (50 ng/ml) for 24 hr, and (d) cells treated with IFN- β for 48 hr with actinomycin D added for the last 24 hr of culture. (e) Bar graph representing the apoptosis of peripheral blood mononuclear cells (PBMC) following culture with IFN- β in the presence or absence of actinomycin D. Data are representative of seven independent experiments. *P < 0.05; **P < 0.001 when compared with cells that were cultured with medium alone; $\Delta P < 0.05$ compared with cultured with actinomycin D alone.

(a)

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Figure 6. Flow cytometric analysis of cell cycle dynamics of CD3⁺ T lymphocytes stimulated with phytohaemagglutinin (PHA) in the presence or absence of interferon (IFN)- β : (a) control, cells cultured in medium alone; (b) cells cultured with IFN- β for 48 hr; (c) cells cultured with PHA for 48 hr and (d) cells cultured with IFN- β and PHA for 48 hr. The figure shows the profile for one representative from six individuals. Regulation of cell cycle progression by IFN-B: (e) G0/G1 phase, (f) S phase and (g) G2 phase. Error bars represent the mean and standard deviation of values for six individuals. *P < 0.05 for the comparison between cells cultured with PHA alone and cells cultured with PHA plus IFN- β .

control cultures dropped from 92.2% at 0 hr to 52.2% at 24 hr, and was 36.7% at 48 hr (Fig. 6e). In CD3⁺ lymphocytes that were cultured with IFN- β (1000 IU/ml) and PHA (10 µg/ml), the percentage of cells in G0/G1 decreased from 91.7% at 0 hr to 63.9% at 24 hr and reduced further to 50.3% at 48 hr (Fig. 6e; P < 0.05compared with cells that did not receive IFN- β , but were cultured with PHA). Also, the percentage of cells in G2 decreased from 24.9% when cultured with PHA alone to 19.65% when IFN- β was added with PHA (Fig. 6g,c,d; P < 0.05). The percentages of cells entering the S phase

in cells that were treated with PHA and IFN- β were also lower compared with cells treated with PHA alone (Fig. 6c,d,f). These results show that, in the presence of PHA, IFN- β induces cell cycle arrest at G0/G1 and decreases the transition to the S phase, and thereby decreases the number of cells in the G2 phase.

Discussion

Using microarray techniques, complemented by real-time RT-PCR and western blot analyses, we show that IFN- β is

capable of the activation of a number of genes involved in the p53 signalling pathway in human PBMC. The proteins that were activated downstream of p53 by IFN- β in our study are to some degree similar to those previously described as being activated by genotoxic stress.^{21,31,32} These include genes that control apoptosis, such as PUMA, NOXA and Bax, and those that induce cell cycle arrest, such as p21 and Sestrin 2. However, unlike the induction of apoptosis that follows activation of p53 after genotoxic stress, IFN- β induces cell cycle arrest in activated lymphocytes. The addition of IFN- β increased the sensitivity of lymphocytes to apoptosis in the presence of Act D. These observations suggest that DNA damage or other additional signals of cellular stress or damage may be necessary for IFN- β to mediate apoptosis in human lymphocytes.

The prevailing view regarding cell lines is that an increase in the constitutive levels of p53 allows time for DNA repair by inducing cell cycle arrest, or instructs the initiation of the cell death if the damage appears irreparable. In our study, the addition of IFN- β to PBMC cultured with PHA restricted the transition of cells from the G0/G1 phase to the S phase (induction of cell cycle arrest) and reduced the number of cells in the G2 phase. Inhibition of the transition from the G1 phase to the S phase involves the activation of a number of genes, of which p21 has been most extensively studied and is a key molecule involved in inhibiting cyclin-dependent kinase 1/2 (CDK1/2)³³ The 12-fold increase in the expression of p21 suggests that this protein, along with other genes that regulate cell cycle arrest, as shown in Table 3, is critical for impeding the transition from G0/G1 to S in cells cultured with IFN- β . However, although a number of genes involved in the apoptotic process, such as BAX, PUMA and NOXA, were up-regulated, the cell death programme was not initiated.

The answer to the fundamental question of how activation of p53 leads to either cell cycle arrest or apoptosis is unclear, especially in light of the finding that induction and activation of the p53 signal pathway are not the result of double-stranded DNA breaks such as are seen with IFN- β . One possibility might relate to the activation of different isoforms of p53. Studies on tumour cell lines showed that transcription of p53 was regulated by a single promoter, producing the full-length transcript and two isoforms.³⁴ More recently, an internal promoter of p53 was described, and at least six additional isoforms, some of which act to interfere with the transcription of the full-length protein, have been described.³⁵ We have shown that IFN- β induces the expression of the fulllength and beta/gamma isoforms of p53 in PBMC. We also observed that the pattern of induction of the fulllength and beta/gamma isoforms seen following stimulation with IFN- β is distinct and different from that seen following genotoxic stress, which predominantly induces full-length p53 only. Thus, the expression of different isoforms of p53 induced by genotoxic injury or IFN- β may also alter the potency of the expression of target genes, which would skew the response towards either cell cycle arrest or apoptosis.

Another possibility might relate to the ability of p53 to bind additional transcription factors and recruit them to the promoter regions of p53 target genes.^{36–39} A study examining the binding of p53 to different DNA-binding sites in yeast and mammalian systems showed a difference in the ability of p53 to bind sites derived from genes involved in cell cycle arrest and/or DNA repair when compared with genes regulating mitochondrial apoptotic pathways.⁴⁰ These results suggest that, whereas only the binding site sequences are required for p53-dependent activation of the cell cycle arrest genes, additional transcription factors are needed for the induction and expression of many of the pro-apoptotic genes. Hence, recruitment of additional transcription factors to p53 targeted genes may differ between cells cultured with IFN- β and cells subjected to genotoxic stress. Activation of haematopoietic zinc finger protein (Hzf), a p53 target protein, results in the transactivation of pro-arrest genes over that of pro-apoptotic genes,⁴¹ and may be favoured in cells stimulated with IFN- β .

Cellular levels of p53 are regulated tightly at the levels of transcription, post-translational modification and degradation. The ISRE that is present in the promoter region of the p53 gene binds to the heterotrimeric complex consisting of STAT1 and STAT2, thereby regulating the transcription of p53.^{19,42} Our studies showed a rapid induction of STAT1 and STAT2 mRNA and proteins following culture with IFN- β . Considering that the induction of both p53 mRNA and protein was modest at 24 hr and significant at 48 hr, this suggests that the initial amplification of STAT1 and STAT2 is necessary for optimal transcription of the p53 gene. Post-translational modifications of p53 are a critical step in the regulation of cellular levels of p53. In normal resting cells, p53 is rapidly degraded following binding to MDM2, thus ensuring cell integrity.⁴³ IFN- β appears to regulate the expression of p53 both at transcription, by increasing mRNA levels, and also at degradation, by increasing the levels of MDM2. Although the increase in the amount of p53 protein in IFN- β -activated cells may be modest, the cellular consequences of activation of downstream targets, especially p21, and the induction of cell cycle arrest were significant.

Our study indicates additional mechanisms by which IFN- β may provide therapeutic benefits in human disease. Although the potency and kinetics of p53 induction varied among donors, all donor cells showed an increase in p53 expression 48 hr after culture with IFN- β . In autoimmune diseases such as multiple sclerosis, by activating p53 targeted genes, IFN- β can induce cell cycle arrest and thereby restrict the expansion of putative autoreactive lymphocytes. Whether the p53 response governs the optimal clinical response is at present not known. In patients being treated for hepatitis caused by hepatitis C virus, activation of the p53 pathway may dictate the antiviral response in hepatocytes. Acting as an adjuvant, IFN- β , by inducing p53-related pro-apoptotic genes, can enhance the actions of chemotherapeutic drugs in inducing cell death and improve outcomes in the treatment of human neoplastic diseases.

Acknowledgements

This study was supported by a postdoctoral fellowship award from the National MS Society (FG 1737A1/1) to FZ and by an investigator originated grant from Serono Inc, and the William Weaver Fund.

Disclosures

Neither author has any conflict of interest to disclose.

References

- de Weerd NA, Samarajiwa SA, Hertzog PJ. Type I interferon receptors: biochemistry and biological functions. J Biol Chem 2007; 282:20053-7.
- 2 Pestka S. The interferons: 50 years after their discovery, there is much more to learn. J Biol Chem 2007; 282:20047–51.
- 3 Takaoka A, Yanai H. Interferon signalling network in innate defence. *Cell Microbiol* 2006; **8**:907–22.
- 4 Uze G, Schreiber G, Piehler J, Pellegrini S. The receptor of the type I interferon family. *Curr Top Microbiol Immunol* 2007; **316**:71–95.
- 5 Darnell JE Jr, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 1994; **264**:1415–21.
- 6 Levy DE, Darnell JE Jr. Stats: transcriptional control and biological impact. *Nat Rev Mol Cell Biol* 2002; **3**:651–62.
- 7 Moriyama M, Arakawa Y. Treatment of interferon-alpha for chronic Hepatitis C. *Expert Opin Pharmacother* 2006; 7:1163– 79.
- 8 Mihelic R, Kaufman JL, Lonial S. Maintenance therapy in multiple myeloma. *Leukemia* 2007; **21**:1150–7.
- 9 Ann Marrie R, Rudick RA. Drug insight: interferon treatment in multiple sclerosis. *Nat Clin Pract Neurol* 2006; **2**:34–44.
- 10 Juang SH, Wei SJ, Hung YM, Hsu CY, Yang DM, Liu KJ, Chen WS, Yang WK. IFN-beta induces caspase-mediated apoptosis by disrupting mitochondria in human advanced stage colon cancer cell lines. *J Interferon Cytokine Res* 2004; **24**:231–43.
- 11 Oehadian A, Koide N, Mu MM, Hassan F, Islam S, Yoshida T, Yokochi T. Interferon (IFN)-beta induces apoptotic cell death in DHL-4 diffuse large B cell lymphoma cells through tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *Cancer Lett* 2005; **225**:85–92.
- 12 Wandinger KP, Lunemann JD, Wengert O *et al.* TNF-related apoptosis inducing ligand (TRAIL) as a potential response marker for interferon-beta treatment in multiple sclerosis. *Lancet* 2003; **361**:2036–43.

- 13 Wandinger KP, Sturzebecher CS, Bielekova B, Detore G, Rosenwald A, Staudt LM, McFarland HF, Martin R. Complex immunomodulatory effects of interferon-beta in multiple sclerosis include the upregulation of T helper 1-associated marker genes. *Ann Neurol* 2001; **50**:349–57.
- 14 Zipp F. Apoptosis in multiple sclerosis. Cell Tissue Res 2000; 301:163-71.
- 15 Sharief MK, Semra YK, Seidi OA, Zoukos Y. Interferon-beta therapy downregulates the anti-apoptosis protein FLIP in T cells from patients with multiple sclerosis. *J Neuroimmunol* 2001; 120:199–207.
- 16 Sharief MK, Semra YK. Upregulation of the inhibitor of apoptosis proteins in activated T lymphocytes from patients with multiple sclerosis. *J Neuroimmunol* 2001; **119**:350–7.
- 17 Weinstock-Guttman B, Bhasi K, Badgett D *et al.* Genomic effects of once-weekly, intramuscular interferon-beta 1a treatment after the first dose and on chronic dosing: relationships to 5-year clinical outcomes in multiple sclerosis patients. *J Neuroimmunol* 2008; **205**:113–25.
- 18 Hilpert J, Beekman JM, Schwenke S *et al.* Biological response genes after single dose administration of interferon beta-1b to healthy male volunteers. *J Neuroimmunol* 2008; 199:115–25.
- 19 Takaoka A, Hayakawa S, Yanai H et al. Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. *Nature* 2003; 424:516–23.
- 20 O'Doherty C, Villoslada P, Vandenbroeck K. Pharmacogenomics of Type I interferon therapy: a survey of response-modifying genes. *Cytokine Growth Factor Rev* 2007; **18**:211–22.
- 21 Vousden KH, Lane DP. p53 in health and disease. *Nat Rev Mol Cell Biol* 2007; **8**:275–83.
- 22 Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature* 2000; **408**:307–10.
- 23 Kastan MB. Wild-type p53: tumors can't stand it. *Cell* 2007; **128**:837–40.
- 24 Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, Cordon-Cardo C, Lowe SW. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 2007; **445**:656–60.
- 25 Martins CP, Brown-Swigart L, Evan GI. Modeling the therapeutic efficacy of p53 restoration in tumors. *Cell* 2006; **127**:1323–34.
- 26 Munoz-Fontela C, Macip S, Martinez-Sobrido L, Brown L, Ashour J, Garcia-Sastre A, Lee SW, Aaronson SA. Transcriptional role of p53 in interferon-mediated antiviral immunity. *J Exp Med* 2008; 205:1929–38.
- 27 Alberti A, Boccato S, Vario A, Benvegnu L. Therapy of acute hepatitis C. *Hepatology* 2002; **5**(Suppl. 1):S195–200.
- 28 Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003; 4:249–64.
- 29 Thomas PD, Campbell MJ, Kejariwal A *et al.* PANTHER: a library of protein families and subfamilies indexed by function. *Genome Res* 2003; **13**:2129–41.
- 30 Kleeff J, Kornmann M, Sawhney H, Korc M. Actinomycin D induces apoptosis and inhibits growth of pancreatic cancer cells. *Int J Cancer* 2000; 86:399–407.
- 31 Fridman JS, Lowe SW. Control of apoptosis by p53. Oncogene 2003; 22:9030–40.

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- 32 Budanov AV, Sablina AA, Feinstein E, Koonin EV, Chumakov PM. Regeneration of peroxiredoxins by p53-regulated sestrins, homologs of bacterial AhpD. *Science* 2004; **304**:596–600.
- 33 Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 1993; 75:805–16.
- 34 Baumbusch LO, Myhre S, Langerod A *et al.* Expression of fulllength p53 and its isoform Deltap53 in breast carcinomas in relation to mutation status and clinical parameters. *Mol Cancer* 2006; **5**:47.
- 35 Bourdon JC, Fernandes K, Murray-Zmijewski F, Liu G, Diot A, Xirodimas DP, Saville MK, Lane DP. p53 isoforms can regulate p53 transcriptional activity. *Genes Dev* 2005; **19**:2122–37.
- 36 Vousden KH. Outcomes of p53 activation spoilt for choice. J Cell Sci 2006; 119:5015–20.
- 37 Riley T, Sontag E, Chen P, Levine A. Transcriptional control of human p53-regulated genes. Nat Rev Mol Cell Biol 2008; 9:402– 12.

- 38 Weinberg RL, Veprintsev DB, Bycroft M, Fersht AR. Comparative binding of p53 to its promoter and DNA recognition elements. J Mol Biol 2005; 348:589–96.
- 39 Koutsodontis G, Vasilaki E, Chou WC, Papakosta P, Kardassis D. Physical and functional interactions between members of the tumour suppressor p53 and the Sp families of transcription factors: importance for the regulation of genes involved in cell-cycle arrest and apoptosis. *Biochem J* 2005; **389**:443–55.
- 40 Qian H, Wang T, Naumovski L, Lopez CD, Brachmann RK. Groups of p53 target genes involved in specific p53 downstream effects cluster into different classes of DNA binding sites. *Oncogene* 2002; **21**:7901–11.
- 41 Das S, Raj L, Zhao B, Kimura Y, Bernstein A, Aaronson SA, Lee SW. Hzf Determines cell survival upon genotoxic stress by modulating p53 transactivation. *Cell* 2007; 130:624–37.
- 42 Vilcek J. Boosting p53 with interferon and viruses. *Nat Immunol* 2003; **4**:825–6.
- 43 Lavin MF, Gueven N. The complexity of p53 stabilization and activation. *Cell Death Differ* 2006; **13**:941–50.