GENE EXPRESSION PROFILING OF THE HEPATIC TRANSCRIPTOME IN THE PRESENCE OF TNF-α.

INSTITUTE OF GENOMICS AND INTEGRATIVE BIOLOGY

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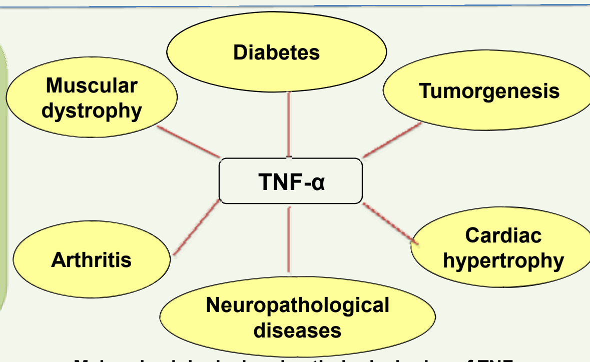
Diabetes mellitus, often simply termed Diabetes, is a syndrome characterized by disordered metabolism and high blood sugar. It is caused due to low levels of insulin hormone or from abnormal resistance to insulin in its target tissues. World Health Organization estimates that India will alone have 79.4 million diabetic patients in 2030. One of its major form Type 2 diabetes, is often associated with obesity, hypertension, elevated cholesterol and metabolic syndrome. Changes in life style, such as consumption of high-calorie diet and lack of exercise, have increased the global prevalence not only of diabetes but also of obesity. Type 2 diabetes is characterized by insulin resistance in target tissue, occurs due to several reasons and one of them being the proinflammatory cytokine, TNF-α. It is also known as the link between diabetes and obesity. High levels of TNF-α interfere with insulin signaling to cause the effect and to further investigate into the situation, gene transcription profiling was examined in control and TNF-α treated HepG2 cells. Results indicated that TNF-α could significantly alter the expression of a significant number of genes that were identified to be related to lipid and fat metabolism on one hand and to immunoglobulin receptor activity and IgE binding thereby on the other thereby indicating global dysregulation of fat metabolism and compromise in immune defense mechanism(s) within the hepatocyte by TNF-α. Pathway analysis revealed "biosynthesis of steroids" to be most effected. All these indicate TNF-α to be significantly altering the transcriptome profiling within HepG2 cells with genes involved in lipid and steroid metabolism being the most favoured and this could explain one of the underlying mechanisms of TNF-α action in the liver.



Major physiological and pathological roles of TNF-α

Genes altered by TNF-α treatment in HepG2 cells.

Gene Symbol	Fold Change	Gene ID	Description	p Val
Cell adhesion				
LAMC1	-1.4	200770_s_at	Laminin, Gamma 1 [Formerly Lamb2]	0.01
TAOK2	-1.6	204877_s_at	Tao Kinase 2	0.04
FGFRL1	-1.5	223321_s_at	Fibroblast Growth Factor Receptor-Like 1	0.03
DGCR6L	1.4	208024_s_at	Digeorge Syndrome Critical Region Gene 6-Like	0.04
CXADR	1.5	226374_at	Coxsackie Virus And Adenovirus Receptor	0.03
Regulation of biological process				
PUM1	-1.8	201165_s_at	Pumilio Homolog 1 [Drosophila]	0.03
CEBPA	-1.7	204039_at	CCAAT/Enhancer Binding Protein [C/EBP], Alpha	0.006
BRPF1	-1.6	204481_at	Bromodomain And PHD Finger Containing, 1	0.04
STARD10	1.5	232322_x_at	Start Domain Containing 10	0.04
CASZ1	1.5	220015_at	Castor Homolog 1, Zinc Finger [Drosophila]	0.03
GTF2H3	1.5	222104_x_at	General Transcription Factor LJH, Polypeptide 3, 34kDa	0.03
ATF5	-1.5	204998_s_at	Activating Transcription Factor 5	0.01
SA[illegible]	1.7	1556590_s_at	Chromosome 11 Open Frame 23	0.02
Signal transduction				
FCER1G	-1.6	204232_at	Fc Fragment Of IgE, High Affinity I, Receptor For; Gamma Polypeptide	0.03
RASSF7	-1.7	204927_at	Ras Association [RALGDS/AF-6] Domain Family7	0.04
NR1H4	-1.7	206340_at	Nuclear Receptor Subfamily 1, Group H, Member 4	0.009
MKNK2	1.8	223199_at	MAP Kinase Interacting Serine/Threonine Kinase 2	0.005
RGL3	-1.8	228877_at	Ral Guanine Nucleotide Dissociation Stimulator-Like 3	0.01
TBL1X	1.7	201867_s_at	Transducin [Beta]-Like 1X-Linked	0.03
RYK	1.6	202853_s_at	RYK Receptor-Like Tyrosine Kinase	0.04
HIST1H4B	1.9	205967_at	H4 Histone, Family 2	0.01
NFKB2	1.5	209636_at	Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-Cells 2	0.002
DDX54	1.5	219111_s_at	DEAD [Asp-Glu-Ala-Asp] Box Polypeptide 54	0.01
IL22RA1	1.5	220056_at	Interleukin 22 Receptor, Alpha 1	0.03
GSG2	1.6	223759_s_at	Germ Cell Associated 2 [Haspin]	0.04
TNFRSF14	1.5	209354_at	Tumor Necrosis Factor Receptor Super Family,Member14[Herpesvirus Entry Mediator]	0.02
MED30	1.7	227786_at	Mediator complex subunit 30	0.03
KISS1R	1.7	242517_at	KISS1 Receptor	0.01
Development				
TGIF1	-1.4	1566901_at	TGFB-induced factor homeobox 1	0.03
JRKL	1.8	206734_at	Jerky Homolog-Like [Mouse]	0.04
Response to stimulus				
HSPA1B	-1.9	200799_at	Heat Shock 70kDa Protein 1A	0.01
ORM2	-1.5	214465_at	Orosomucoid 2	0.005
MST1	-1.5	216320_x_at	Macrophage Stimulating 1 [Hepatocyte Growth Factor-Like]	0.02

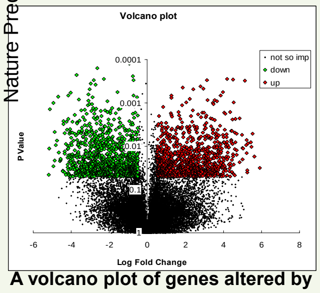
Gene Symbol	Fold Change	Gene ID	Description	p Val
HIST2H3A	-2	222067_x_at	Histone cluster 2, H3a	0.01
C4B	-1.5	235856_at	Complement Component 4A [Rodgers Blood Group]	0.02
Death				
TM2D1	-2.4	236524_at	TM2 Domain Containing 1	0.009
PDCD7	1.4	231809_x_at	Programmed Cell Death 7	0.02
Transport				
FLJ11506	-2	202852_s_at	Hypothetical Protein Flj11506	0.04
AP3B1	-1.7	203141_s_at	Adaptor-Related Protein Complex 3, Beta1 Subunit	0.03
SLC6A12	-3.2	206058_at	Solute Carrier Family 6 [Neurotransmitter Transporter, Betaine/GABA], Member 12	0.04
RAB11FIP1	-1.8	219681_s_at	Rab11 Family Interacting Protein 1 [Class I]	0.04
STXBP5	-1.5	226794_at	Syntaxin Binding Protein 5 [Tomosyn]	0.03
AP1S2	-1.4	228415_at	Adaptor-Related Protein Complex 1, Sigma2 Subunit	0.04
AQP3	-2.9	39248_at	Aquaporin 3 [Gill Blood Group]	0.02
LOC442285	1.5	1553148_a_at	Hypothetical LOC442285	0.003
SRGAP2	1.7	1568957_x_at	Slit-Robo GTPase Activating Protein 2	0.04
GOSR2	1.4	210009_s_at	Golgi Snap Receptor Complex Member 2	0.02
SEC24A	1.4	212900_at	Sec24 Related Gene Family, Member A [S. Cerevisiae]	0.03
HCN3	1.7	222078_at	Hyperpolarization Activated Cyclic Nucleotide-Gated Potassium Channel 3	0.03
SLC43A2	1.9	226629_at	Solute Carrier Family 43, Member 2	0.04
COX18	1.4	227134_at	COX18 cytochrome c oxidase assembly homolog (S. cerevisiae)	0.02
VPS41	1.7	235625_at	Vacuolar Protein Sorting 41 [Yeast]	0.003
KCTD20	1.6	228299_at	potassium channel tetramerisation domain containing 20	0.01
KIF3A	1.7	228680_at	Kinesin Family Member 3A	0.04
Cell proliferation				
TIMP1	-1.5	201666_at	TIMP Metallopeptidase Inhibitor 1	0.04
UBR5	1.5	1555888_at	ubiquitin protein ligase E3 component n-recognin 5	0.01
NRD1	2.1	242235_x_at	Nardilysin [N-Arginine Dibasic Convertase]	0.005
Homeostasis				
PDIA4	-1.9	208658_at	Protein Disulfide Isomerase Family A,Member4	0.01
PDIA4	-1.9	211048_s_at	Protein Disulfide Isomerase Family A,Member4	0.03
CALR	-1.6	214315_x_at	Calreticulin	0.002
Gene expression				
POLR2J3	-1.8	1552621_at	DNA Directed RNA Polymerase II Polypeptide J-Related Gene	0.03
LOC113179	-1.6	1553968_a_at	Hypothetical Protein BC011824	6.9E-05
RGNEF	-2.1	1554003_at	Rho-Guanine Nucleotide Exchange Factor	0.03
ATF7IP2	-1.9	228381_at	Activating Transcription Factor 7 Interacting Protein 2	0.008
LOC148203	-1.5	229700_at	Hypothetical Protein LOC148203	0.03
SEPSECS	-1.7	231730_at	Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase	0.04
C14ORF172	-1.6	52741_at	Chromosome 14 Open Frame 172	0.03

Gene Symbol	Fold Change	Gene ID	Description	p Val
Protein metabolism				
STOM	-1.7	201061_s_at	Stomatin	0.03
UBE2J1	-1.6	222435_s_at	Ubiquitin-Conjugating Enzyme E2,J1[UBC6 Homolog,Yeast]	0.03
SCRN3	-1.5	222849_s_at	Secernin 3	0.04
7-Sep	1.7	1565823_at	Septin 7	0.03
TRIM5	2	210705_s_at	Tripartite Motif-Containing 5	0.03
UBE2NL	1.7	217393_x_at	Ubiquitin-Conjugating Enzyme E2N-Like	0.04
PSME3	-1.9	200987_x_at	Proteasome [Prosome, Macropain] Activator Subunit 3 [PA28 Gamma; Ki]	0.04
HSP90B1	-2.1	216449_x_at	Heat Shock Protein 90kDa Beta [Grp94], Member 1	0.03
IMPACT	1.6	218637_at	Impact Homolog [Mouse]	0.03
Carbohydrate metabolism				
AKR1B1	-1.4	201272_at	Aldo-Keto Reductase Family 1, Member B1 [Aldose Reductase]	0.03
GNPDA2	-1.8	227022_at	Glucosamine-6-Phosphate Deaminase 2	0.04
PDK3	-1.8	230085_at	Pyruvate Dehydrogenase Kinase, Isozyme 3	0.04
IDH2	1.5	210046_s_at	Isocitrate Dehydrogenase 2 [NADP+], Mitochondrial	0.03
Amino acid metabolism				
C1ORF69	-1.6	236629_at	Chromosome 1 Open Frame 69	0.01
PAH	1.8	242375_x_at	Phenylalanine Hydroxylase	0.03
Nucleic Acid metabolism				
MTHFS	1.7	210242_x_at	5,10-Methenyltetrahydrofolate Synthetase [5-Formyltetrahydrofolate Cyclo-Ligase]	0.03
EP400	-1.5	212376_s_at	Trinucleotide Repeat Containing 12	0.04
SIRT6	-1.5	219613_s_at	Sirtuin [Silent Mating Type Information Regulation 2 Homolog] 6 [S. Cerevisiae]	0.03
ELF5	-1.7	220625_s_at	E74-Like Factor 5 [ETS Domain Transcription Factor]	0.04
DNMT3B	-1.9	220668_s_at	DNA [Cytosine-5-]-Methyltransferase 3 Beta	0.01
NDUFS1	1.7	1559691_at	NADH Dehydrogenase [Ubiquinone] Fe-S Protein 1, 75kDa [NADH-Coenzyme Q Reductase]	0.02
DHFR	1.5	202532_s_at	Dihydrofolate Reductase	0.04
TOX3	1.5	215108_x_at	TOX high mobility group box family member 3	0.02
Lipid metabolism				
PCYT1A	-1.4	204209_at	Phosphate Cytidylyltransferase 1, Choline, Alpha	0.002
FDPS	1.6	201275_at	Farnesyl Diphosphate Synthase [Farnesyl Pyrophosphate Synthetase, Dimethylallyl Transtransferase, Geranyltranstransferase]	0.006
PAFAH2	2	205232_s_at	Platelet-Activating Factor Acetyl-Hydrolase 2, 40kDa	0.01
FADS1	1.7	208964_s_at	Fatty Acid Desaturase 1	9.5E-05
SQLE	2.5	213577_at	Squalene Epoxidase	0.002
SLC27A5	2	219733_s_at	Solute Carrier Family 27 [Fatty Acid Transporter], Member 5	0.02
EBP	1.7	202735_at	Emopamil Binding Protein [Sterol Isomerase]	0.03

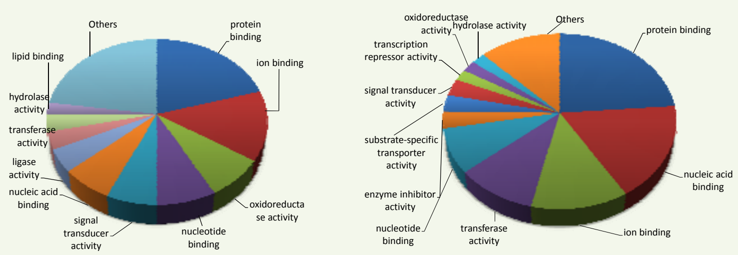
Gene Symbol	Fold Change	Gene ID	Description	p Val
HSD17B7	1.7	220081_x_at	Hydroxysteroid [17-Beta] Dehydrogenase 7	0.04
Electron transport				
GCDH	-1.6	203500_at	Glutaryl-Coenzyme A Dehydrogenase	0.02
DHRS4	1.4	218021_at	Dehydrogenase/Reductase [SDR Family] Member 4	0.01
Others				
YTHDF3	-2.1	1564053_a_at	YTH Domain Family, Member 3	0.04
GOLGA8B	-1.8	213650_at	Golgi Autoantigen, Golgin Subfamily A, 8B	0.02
TMEM63A	-1.5	202700_s_at	Transmembrane Protein 63A	0.01
C17orf91	-1.7	214696_at	Chromosome 17 open reading frame 91	0.03
XTP3TPA	-1.7	218069_at	XTP3-Transactivated Protein A	0.04
KANK2	-2	218418_s_at	KN motif and ankyrin repeat domains 2	0.04
C12ORF49	-2.3	218867_s_at	Chromosome 12 Open Frame 49	0.03
ATG2B	-1.4	219164_s_at	ATG2 autophagy related 2 homolog B (S. cerevisiae)	0.02
C2orf43	-1.9	222193_at	Chromosome 2 open reading frame 43	0.02
TMEM50B	-1.6	228001_at	Transmembrane Protein 50B	0.04
ZCCHC2	-1.8	233425_at	Zinc Finger, CCHC Domain Containing 2	0.006
C8ORF38	-1.8	236766_at	Chromosome 8 Open Frame 38	0.03
C5orf28	-1.7	238635_at	chromosome 5 open reading frame 28	0.04
FAM13C1	1.9	1554547_at	Family With Sequence Similarity13,Member C1	0.01
LOC51336	2.2	208154_at	Mesenchymal Stem Cell Protein DSCD28	0.04
TXNDC9	2.1	1554047_at	Thioredoxin Domain Containing 9	0.004
CEP135	1.5	206003_at	Centrosomal Protein 135kDa	0.02
LOC171220	1.5	211325_x_at	Destrin-2 Pseudogene	0.003
KIAA0913	1.6	212359_s_at	KIAA0913	0.02
HCG2P7	1.5	216229_x_at	HLA Complex Group 2 Pseudogene 7	0.04
MALAT1	1.5	224567_x_at	Metastasis Associated Lung Adenocarcinoma Transcript 1 [Non-Coding RNA]	0.02
C12ORF23	1.6	224759_s_at	Chromosome 12 Open Frame 23	0.02
KDELC2	1.7	225128_at	KDEL [Lys-Asp-Glu-Leu] Containing 2	0.005
HCG12	1.5	227980_at	HLA Complex Group 12	0.03
C10ORF58	1.5	228155_at	Chromosome 10 Open Frame 58	0.04
PHACTR4	3.3	233319_x_at	Phosphatase And Actin Regulator 4	0.03
PGBD2	1.7	238004_at	PIGGYBAC Transposable Element Derived 2	0.047
FAM114A2	-1.5	218588_s_at	Family with sequence similarity 114, member A2	0.046
LYSMD4	-1.7	228954_at	LYSM, Putative Peptidoglycan-Binding, Domain Containing 4	0.01
HHLA3	-2	234665_x_at	HERV-H LTR-Associating 3	0.03
MYO19	-1.6	236022_at	Myosin XIX	0.047
LRRC8E	-1.42	239433_at	Leucine Rich Repeat Containing8 family MemberE	0.007
ZDHHC21	-2	243835_at	Zinc Finger, DHHC-Type Containing 21	0.046

GO term and Gene Symbol*	Transcription Factors	Probability**
Metabolism		
SQLE, EBP, TIMP1, DHRS4, UBE2J1,	FOXJ2	3.31E-156
UBE2NL, PAH, HSP90B1, GCDH	FOXD3	5.75E-136
DHFR, FADS, SIRT6, AKR1B1	HFH-3	2.42E-128
C4B, SLC25A5	IRF-1	4.06E-108
	SREBP-1	1.41E-53
	E47	1.17E-52
	MEF-2	6.67E-42
	AREB6	9.87E-41
	ARP-1	1.51E-35
Signal Transduction		
TBL1X, RYK, DDX54, TNFRSF14,	FOXJ2	3.04E-58
KISSIR, FCER1G, NFkB2	FOXD3	5.50E-58
	IRF-1	3.42E-52
	HFH-3	8.90E-48
	SREBP-1	2.71E-19
	CHOP:C/EBPalpha	2.51E-14
	ARP-1	1.26E-13
	Freac-7	2.98E-12
	E47	2.78E-11
	MEF-2	5.14E-11
Regulation of biological processess		
CASZ1, PUM1, C4B, GTF2H3 ATF5,	MEF-2	1.06E-14
	CHOP:C/EBPalpha	1.93E-14
	FOXJ2	6.57E-13
	AREB6	3.25E-12
	SREBP-1	3.96E-12
	ARP-1	1.69E-11
	p300	2.32E-11
	FOXD3	3.39E-11
	SREBP-1	3.09E-10
	NF-E2	3.89E-10

Over representation of conserved transcription factor binding sites within putatively co-regulated genes.

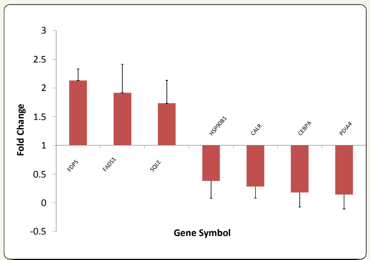


A volcano plot of genes altered by TNF-α in HepG2 cells

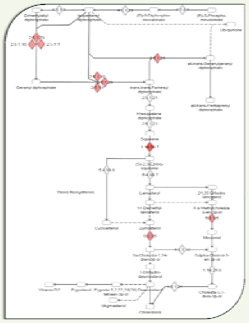

Others
protein binding
ion binding
lipid binding
hydrolase activity
transferase activity
ligase activity
nucleic acid binding
signal transducer activity
nucleotide binding
oxidoreductase activity
transcription repressor activity
signal transducer activity
substrate-specific transporter activity
enzyme inhibitor activity
nucleotide binding
transferase activity
ion binding
nucleic acid binding

A. 67 genes up-regulated by TNF-α B. 73 genes down-regulated by TNF-α

Classification of TNF-α regulated genes into functional groups



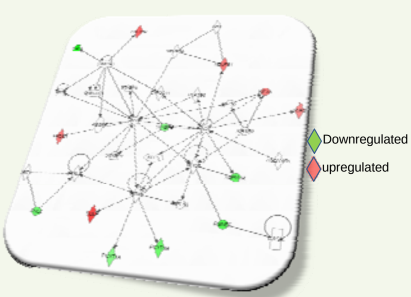
Validation of microarray gene expression data by Real Time PCR



"Biosynthesis of steroids" identified as the top canonical pathway altered by TNF-α in HepG2 cells

Conclusion

- ❖As many as 140 genes were significantly altered by TNF-**α.** Pathway analysis identified the biosynthesis of steroids and cholesterol to be the most favored
- ❖Signatures of conserved transcription factor binding sites were identified in genes of similar GO functional term and within the same cluster.
- ❖Over represented genes upregulated by TNF-**α** consisted of several Gene ontology terms related to lipid and fat metabolism
- ❖Within the down-regulated category, those involved in varied aspects of the immune response were over-represented in the GO classes of both biological processes and molecular function



Interacting network among genes altered by TNF-α in HepG2 cells

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