

More Related Gene Pathways to Vincristine-Induced Death Events in a Human T-Acute Lymphoblastic Leukemia Cell Line

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is common in children but rare in adults. Vincristine (VCR) is one of the drugs used at the beginning of treatment. Some genes are resistant to VCR in B-ALL. **Methods:** Here, we examined the effect of VCR on gene expression changes in a T-ALL cell line, Jurkat. The MTT method was used to determine the IC₅₀ in Jurkat cells treated with different concentrations of VCR for 48 and 72 hours. Total RNA was isolated from the cells and cDNA was prepared. The Human Cancer Drug Target PCR Array kit was used to evaluate the 84 gene expression changes in Jurkat cells. Protein-protein interaction was analyzed by STRING software.

Results: We identified 66 differentially expressed genes as comparison to untreated cells. The response to VCR-induced apoptotic events was remarkable in the pathways of heat shock protein, topoisomerases, protein kinases, cathepsins and cell cycle. In other pathways, there were resistant genes as well as sensitive genes to VCR treatment. Some proteins like HSP90AA1 and ESR1 had determining associations with other proteins.

Conclusions: The results suggest VCR target genes in T-ALL cells may be beneficial biomarkers for ALL treatment and can be used to select appropriate synergistic drugs for VCR.

Keywords: ALL, Gene expression profile, Jurkat, PCR array, Vincristine.

Introduction

ALL is a malignancy of lymphoid cells and is common in children with an incidence of 80% (1). ALL is rare but fatal in adults (2). New progress for the treatment of ALL has increased survival in children (3). However, the success of these therapies in adults decreases in older ages (2).VCR. corticosteroids anthracycline are the primary drugs used in chemotherapy of ALL by induction therapy for complete healing of the disease (1). Some patients with ALL (mainly B-ALL) exhibit resistance to anti-leukemic drugs such as VCR (4) and also *in vitro* resistance to VCR and other drugs has been shown as lethal concentration (LC₅₀) in leukemic cells isolated from children diagnosed with B linage ALL (5). The genetic

basis of resistance to chemotherapy with prednisolone (PRE), VCR, asparaginase (ASP), or daunorubicin (DNR) was determined in other studies (4, 5). In another study, signal transduction genes related to drug resistance were identified in children with ALL as *ex vivo*. The drugs included PRE, VCR, ASP, etoposide (VP16), fludarabine (FLU) (6).

VCR is a vinca alkaloid isolated from leaves of *Catharanthus roseus* (7) and used for the treatment of various cancers (8). VCR influences cells by inhibiting cell division and establishment of microtubules and results in mitotic arrest (9). VCR can induce apoptosis in some leukemic cell lines (7) such as acute T-lymphoblastic CCRF-CEM.f2, pre-B ALL-697

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and Jurkat. Previous studies showed VCR induced apoptosis by extrinsic and intrinsic pathways in Jurkat cells (10). The most studies investigate the gene profile in VCR- resistant B-ALL cells (4-6). A few studies have explored gene resistant to drugs in T-ALL cells. In a study, gene expression of cells isolated from 7 patients with T-ALL was analyzed. These patients received a four-drug combination of VCR, dexamethasone (DEX), ASP and DNR (3). In another study by Beesley et al. sensitive and resistance pathways to VCR and other antidrugs were determined leukemia microarray data from T-ALL cell lines derived from pediatric ALL bone marrow specimens. A gene model was made based on resistant genes and used for 3 patients in primary cohort. The patients were treated with 10 drugs and. The evaluation was indicated that T-ALL patients to be sensitive to 6-mercaptopurine. The aim of this study was finding the genes and pathways associated with resistance (11). However, there is not a study that especially evaluates gene expression of pathways associated with cancer drug targets in T-ALL cell line or patients treated with VCR. The aim of this study was to determine a gene expression profile of the T-ALL cell line in response to VCR treatment. So, we evaluated gene expression changes of human cancer drug targets in the T-ALL Jurkat cells to identifying relevant pathways to VCR treatment.

Materials and Methods

Cell culture

The human T-lymphoblastic leukemia cell line (Jurkat, cell line number: C121) was purchased from the cell bank of the Pasture Institute (Tehran, Iran). The cell line identified in DNA Typing laboratory of the Pasture Institute (by STR analysis method). Also, the cells were tested for microbial and fungal contaminations, especially mycoplasma contamination, in Microbial Quality Control laboratory of the Pasture Institute. Cells cultured in RPMI-1640 medium (Gibco, USA, 11875093) with supplemented with 10% heatinactivated fetal bovine serum (FBS) (Gibco, A25904DG) penicillin-USA. and

streptomycin (Gibco, USA, 15140122) and incubated at 37 °C in a 5% CO2 atmosphere with 95% humidity. When the cells were obtained from cell bank, first, were cultured and passaged for one month. After that, the cells were harvested in tubes containing DMSO and frizzed and maintained in the nitrogen tank. These cells were cultured and passaged again to perform experiments after about one year.

MTT assay

Cell growth inhibition was measured based on 5-dimethylthiazol-2-yl)-2, diphenyl-tetrazoliumbromide (MTT) assay. 6×10⁴ cells per well of Jurkat were seeded into a 96-well plate and treated with different concentrations of VCR (0.05, 0.1, 0.5 and 1 µg/ml). After 48- and 72-hours incubation at 37 °C, 20 µl of MTT solution (5 mg/ml in PBS) (Sigma, USA, A101161) was added into each well and cells were incubated at 37 °C for 4 hours. After adding DMSO to wells to dissolve the formazan crystals, plates were read using a plate reader at 570 nm against 630 nm. The experiment was performed three times. The 50% inhibitory concentration (IC50) was determined. Cell growth (%) was calculated as following (12):

$$\text{Cell growth (\%)} = \frac{A_{490nm}(sample)}{A_{490nm}(control)} \times 100$$

Isolation of total RNA and cDNA preparation

Jurkat cells (3.5×105 cells/ml) were treated with vincristine (in a concentration based on IC50) for 72 h. Total RNA was extracted from harvested cells using **RNX-Plus** the (Sinaclon, Tehran, Iran, RN7713C). The quality and quantity of RNA were estimated Picodrop the machine by (Picodrop Microliter, USA). Then, RNA treated with RNase- free DNase I, for removing genomic DNA, was converted to cDNA using First Strand cDNA Synthesis kit (Parstous, Iran, A101161; Thermo Fisher Scientific, USA, EN0521) according to manufacturer's instruction.

Gene expression analysis by PCR array

The Human cancer drug target RT² ProfilerTM PCR Array (Qiagen, USA, PAHS-507Z) was used to screen 84 gene expression changes of 17 pathways. Real-time PCR was performed according to the manufacturer's instruction. The diluted cDNA was mixed with RT2 SYBR Green/ROX PCR master mix (Qiagen, USA, PAHS-507Z) and RNase-free water with a final volume of 2700 µl. Subsequently, 25 µl of the reaction mixture was placed in to each well of a 96 well plate and qRT-PCR was carried out on ABI 7300 machine (Applied Biosystems, USA) by a three -step cycling program including one cycle in 95 °C for 10 min, 40 cycles in 95 °C for 15 s and, finally, 60 °C for 1 min. Each experiment was carried out in duplicate (n= 2). The $\Delta\Delta$ Ct method was used for PCR array data analysis. The analysis was performed using the software in the web portal of Qiagene company (http://pcrdataanalysis.sabiosciences.com /pcr/arrayanalysis.php) The data normalized with five housekeeping genes including *B2M*, *HPRT1*, *RPL13A*, *GAPDH*, *ACTB*.

Protein-interaction analysis

We model a protein interaction network to analyze the association between human cancer drug target genes induced with VCR in Jurkat cells. STRING 11.0 software was used to construct protein-protein interaction map of genes.

Statistical analysis

The data are presented as mean±standard error mean (SEM). Graph of MTT assay test was processed with GraphPad prism 6.0.

Results

Cell growth assay

The proliferation percent of Jurkat cells treated with different doses of VCR was examined during 48 and 72 h using MTS assay. Table 1 shows IC₅₀ values VCR in Jurkat cells.

Table 1. IC₅₀ of VCR in Jurkat cells treated for 48 and 72 h.

Time	IC ₅₀ (μg/ml) 95% Confidence Interval
48 h	0.54 (0.2493 to 1.176)
72 h	0.17 (0.1086 to 0.2640)

Gene expression changes

PCR array analysis was performed to identify molecular targets that are affected by VCR in Jurkat cells. Human cancer drug target array results showed that 66 genes of 84 genes had expression levels higher or less than 1.5 in Jurkat cells treated with VCR (0.5 µg/ml) in

comparison to untreated cells, 50 genes were down-regulated, and 16 genes were upregulated (Table 2). In the scatter plot, the central line indicates unchanged gene expression, blue and yellow dots were down-regulated and up-regulated genes, respectively (Fig. 1).

Table 2. The results of the PCR array for Jurkat cells treated with VCR. Values less and more than 1.5 show gene down-regulation and up-regulation as compared to non-treated cells (control group).

UniGene ID	Gene Description	Gene symbol	Fold regula tion
Apoptosis			
Hs.150749	B-cell CLL/lymphoma 2	BCL2	-
Hs.744872	Baculoviral IAP repeat-containing 5	BIRC5	1.95
PI-3 Kinases	& Phosphatases		
Hs.338207	Mechanistic target of rapamycin (serine/threonine kinase)	MTOR	2.49
Hs.736397	Phosphoinositide-3-kinase, class 2, alpha polypeptide	PIK3C2A	-2.78

Gene Profile of VCR Treated T-ALL Cells

Hs.656958	Phosphoinositide-3-kinase, class 3	PIK3C3	-5.35
Hs.553498	Phosphoinositide-3-kinase, catalytic, alpha polypeptide	PIK3CA	-9.09
Growth Fact	ors & Receptors		
Hs.488293	Epidermal growth factor receptor	EGFR	-1.77
Hs.446352	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	ERBB2	1.90
Hs.118681	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	ERBB3	-4.25
Hs.390729	V-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)	ERBB4	-2.13
Hs.11392	C-fos induced growth factor (vascular endothelial growth factor D)	FIGF	-3.43
Hs.594454	Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular permeability factor receptor)	FLT1	-4.92
Hs.646917	Fms-related tyrosine kinase 4	FLT4	2.45
Hs.160562	Insulin-like growth factor 1 (somatomedin C)	1GF1	1.57
Hs.643120	Insulin-like growth factor 1 receptor	IGF1R	-2.48
Hs.700350	Insulin-like growth factor 2 (somatomedin A)	IGF2	2.91
Hs.479756	Kinase insert domain receptor (a type III receptor tyrosine kinase)	KDR	-4.00
Hs.479754	V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	KIT	-5.17
Hs.74615	Platelet-derived growth factor receptor, alpha polypeptide	PDGFRA	-
Hs.509067	Platelet-derived growth factor receptor, beta polypeptide	PDGFRB	-
Drug Metabo	olism		
Hs.391464	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	ABCC1	-1.66
Hs.523836	Glutathione S-transferase pi 1	GSTP1	-1.88
Hs.196384	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	PTGS2	-
Hs.435136	Thioredoxin	TXN	-
Hs.690011	Thioredoxin reductase 1	TXNRD1	-5.95
G Protein Sig	gnaling		
Hs.247077	Ras homolog gene family, member A	RHOA	-2.13
Hs.502876	Ras homolog gene family, member B	RHOB	8.06
Hormone Re	ceptors		
Hs.744830	Estrogen receptor 1	ESR1	2.09
Hs.660607	Estrogen receptor 2 (ER beta)	ESR2	-
Hs.32405	Progesterone receptor	PGR	-10.68
Heat Shock I	Proteins		
Hs.525600	Heat shock protein 90kDa alpha (cytosolic), class A member 1	HSP90AA 1	-2.69
Hs.192374	Heat shock protein 90kDa beta (Grp94), member 1	HSP90B1	-2.95
Receptor Ty	rosine Kinase Signaling		
		AKT1	-
Hs.525622	V-akt murine thymoma viral oncogene homolog 1	AKII	
Hs.525622 Hs.631535	V-akt murine thymoma viral oncogene homolog 1 V-akt murine thymoma viral oncogene homolog 2	AKT1	-

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Hs.520898 Cathepsin B CTSB -1.88 Hs.654447 Cathepsin D CTSD -1.88 Hs.731507 Cathepsin L1 CTSL -3.51 Hs.181301 Cathepsin S CTSS -4.75 Cel cycle Hs.732435 Cyclin-dependent kinase 1 CDK1 -2.49 Hs.19192 Cyclin-dependent kinase 2 CDK2 -1.77 Hs.95577 Cyclin-dependent kinase 4 CDK3 -1.90 Hs.647078 Cyclin-dependent kinase 5 CDK5 -1.90 Hs.184298 Cyclin-dependent kinase 8 CDK8 -6.60 Hs.150423 Cyclin-dependent kinase 9 CDK9 -3.18 Hs.437505 Cell division cycle 25 homolog A (S. pombe) CDC25A - Hs.434551 Mdm2 p53 binding protein homolog (mouse) MDM4 -10.43 Hs.492203 Telomerase reverse transcriptase TERT 1.81 Topoisomerase; Type II Hs.156346 Topoisomerase (DNA) II alpha 170kDa TOP2A -3.56 Hs.47733 Topoisomerase (DNA) II beta 18	Cathepsins			
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Hs.631535 V-akt murine thymoma viral oncogene homolog 2 ATF2 -7.34 Hs.597216 Hypoxia inducible factor 1, alpha subunit (basic helixloop-helix transcription factor) HIF1A -8.01 Hs.521181 Interferon regulatory factor 5 IRF5 -2.37 Hs.618430 Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 NFKB1 - Hs.437460 Tumor protein p53 TP53 - Protein Kinases Hs.250822 Aurora kinase A AURKA -6.81 Hs.442658 Aurora kinase B AURKC -3.03 Hs.98338 Aurora kinase C AURKC -3.60 Hs.592049 Polo-like kinase 1 PLK1 -1.56 Hs.398157 Polo-like kinase 2 PLK2 -8.26 Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, beta PRKCB 1.75 Hs.580351 Protein kinase C, delta PRKCB -3.35 RAS Signaling	Hs.475733	Topoisomerase (DNA) II beta 180kDa	TOP2B	-5.96
Hs.597216 Hypoxia inducible factor 1, alpha subunit (basic helixloop-helix transcription factor) HIF1A -8.01 Hs.521181 Interferon regulatory factor 5 IRF5 -2.37 Hs.618430 Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 NFKB1 - Hs.437460 Tumor protein p53 TP53 - Protein Kinases Hs.250822 Aurora kinase A AURKA -6.81 Hs.442658 Aurora kinase B AURKB -3.03 Hs.98338 Aurora kinase C AURKC -3.60 Hs.592049 Polo-like kinase 1 PLK1 -1.56 Hs.398157 Polo-like kinase 2 PLK2 -8.26 Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.580351 Protein kinase C, epsilon PRKCB -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Transcriptio	n Factors		
Histarrow Hist	Hs.631535	V-akt murine thymoma viral oncogene homolog 2	ATF2	-7.34
Hs.618430 Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 NFKB1 - Hs.437460 Tumor protein p53 TP53 - Protein Kinases Hs.250822 Aurora kinase A AURKA -6.81 Hs.442658 Aurora kinase B AURKB -3.03 Hs.98338 Aurora kinase C AURKC -3.60 Hs.592049 Polo-like kinase 1 PLK1 -1.56 Hs.398157 Polo-like kinase 2 PLK2 -8.26 Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.597216		HIF1A	-8.01
Hs.618430 B-cells 1 TP53 TP53 TP53 TP54 TP55 TP55	Hs.521181	Interferon regulatory factor 5	IRF5	-2.37
Protein Kinases Hs.250822 Aurora kinase A AURKA -6.81 Hs.442658 Aurora kinase B AURKB -3.03 Hs.98338 Aurora kinase C AURKC -3.60 Hs.592049 Polo-like kinase 1 PLK1 -1.56 Hs.398157 Polo-like kinase 2 PLK2 -8.26 Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.618430		NFKB1	-
Hs.250822 Aurora kinase A AURKA -6.81 Hs.442658 Aurora kinase B AURKB -3.03 Hs.98338 Aurora kinase C AURKC -3.60 Hs.592049 Polo-like kinase 1 PLK1 -1.56 Hs.398157 Polo-like kinase 2 PLK2 -8.26 Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.437460	Tumor protein p53	TP53	-
Hs.442658 Aurora kinase B AURKB -3.03 Hs.98338 Aurora kinase C AURKC -3.60 Hs.592049 Polo-like kinase 1 PLK1 -1.56 Hs.398157 Polo-like kinase 2 PLK2 -8.26 Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Protein Kina	ases		
Hs.98338 Aurora kinase C AURKC -3.60 Hs.592049 Polo-like kinase 1 PLK1 -1.56 Hs.398157 Polo-like kinase 2 PLK2 -8.26 Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.250822	Aurora kinase A	AURKA	-6.81
Hs.592049 Polo-like kinase 1 PLK1 -1.56 Hs.398157 Polo-like kinase 2 PLK2 -8.26 Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.442658	Aurora kinase B	AURKB	-3.03
Hs.398157 Polo-like kinase 2 PLK2 -8.26 Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.98338	Aurora kinase C	AURKC	-3.60
Hs.632415 Polo-like kinase 3 PLK3 -1.96 Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.592049	Polo-like kinase 1	PLK1	-1.56
Hs.172052 Polo-like kinase 4 PLK4 -3.51 Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.398157	Polo-like kinase 2	PLK2	-8.26
Hs.531704 Protein kinase C, alpha PRKCA 1.75 Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.632415	Polo-like kinase 3	PLK3	-1.96
Hs.460355 Protein kinase C, beta PRKCB 1.75 Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.172052	Polo-like kinase 4	PLK4	-3.51
Hs.155342 Protein kinase C, delta PRKCD -2.01 Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.531704	Protein kinase C, alpha	PRKCA	1.75
Hs.580351 Protein kinase C, epsilon PRKCE -3.35 RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.460355	Protein kinase C, beta	PRKCB	1.75
RAS Signaling Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.155342	Protein kinase C, delta	PRKCD	-2.01
Hs.37003 V-Ha-ras Harvey rat sarcoma viral oncogene homolog HRAS -	Hs.580351	Protein kinase C, epsilon	PRKCE	-3.35
	RAS Signalia	ng		
Hs.505033 V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog KRAS -2.18	Hs.37003	V-Ha-ras Harvey rat sarcoma viral oncogene homolog	HRAS	-
	Hs.505033	V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	KRAS	-2.18

Gene Profile of VCR Treated T-ALL Cells

Hs.486502	Neuroblastoma RAS viral (v-ras) oncogene homolog	NRAS	-3.05
Histone Dead	eetylases		
Hs.88556	Histone deacetylase 1	HDAC1	-
Hs.744132	Histone deacetylase 11	HDAC11	-
Hs.3352	Histone deacetylase 2	HDAC2	-9.53
Hs.519632	Histone deacetylase 3	HDAC3	-2.23
Hs.20516	Histone deacetylase 4	HDAC4	1.71
Hs.6764	Histone deacetylase 6	HDAC6	-
Hs.200063	Histone deacetylase 7	HDAC7	-
Hs.310536	Histone deacetylase 8	HDAC8	1.62
Poly ADP-Ri	bose Polymerases		
Hs.177766	Poly (ADP-ribose) polymerase 1	PARP1	-1.50
Hs.409412	Poly (ADP-ribose) polymerase 2	PARP2	1.50
Hs.744855	Poly (ADP-ribose) polymerase family, member 4	PARP4	-8.14
Hs.370267	Tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase	TNKS	-2.67
Structural p	rotein		
Hs.158336	Netrin 3	NTN3	12.35

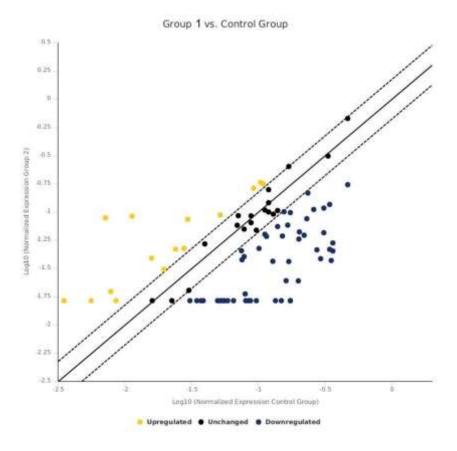


Fig. 1. Effect of VCR on gene expression profiles of Jurkat cells. Eighty-four human cancer drug target genes were evaluated in Jurkat cells treated by VCR in a concentration of $0.5~\mu g/ml$. Scatter plot diagram compares the gene expression changes of cells treated by VCR to control cells (untreated cells). The yellow and blue dots show up-regulated and down-regulated genes, respectively and the black dots are un-changed genes.

Functional and physical interaction network for VCR-induced genes in Jurkat cells

To identify associations between differentially expressed genes in Jurkat cells treated with VCR, a protein interaction network was made using information of the STRING database. In this network, proteins (nods) joint to each other by lines functionally and physically. Totally, 244 putative interactions among 66 proteins were observed that are production of up- and

down- regulated genes (Fig. 2). Among these activated proteins, HSP90AA1 had the most interactions with other proteins of modules. Also, CDK1, PRKCA, RHOA, GRB2, PIK3CA, ERBB2 and ERBB4 interacted with many proteins. Proteins of pathways including drug metabolism, cathepsins and poly ADP-ribose polymerases had the fewer interactions with other pathways.

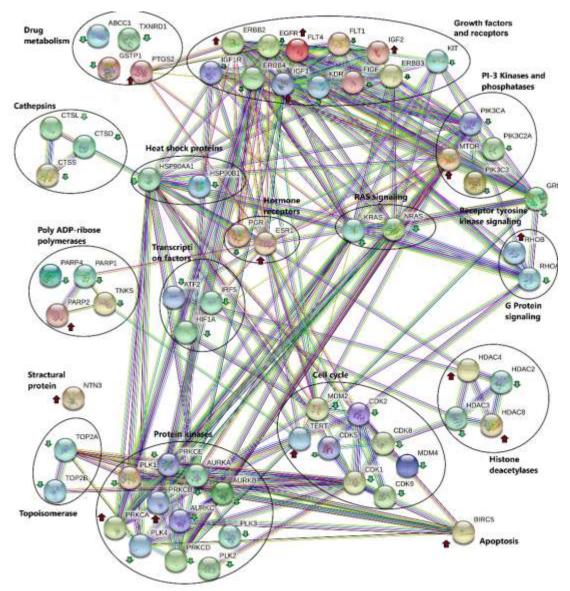


Fig. 2. The cancer functional and physical interaction network among genes induced by VCR in Jurkat cells. Nods and lines represent proteins and protein-protein associations, respectively. The proteins were manually put in clusters based on gene pathways of human cancer drug target PCR array kit. The name of each cluster was labeled above it. Overexpressed and low-expressed proteins are marked with dark red and green arrows, respectively. The network includes 66 proteins and 244 interactions among them. Green, red, blue, black, light blue, brown and purple lines indicate neighborhood, gene fusion, co-occurrence, co-expression, experiments, database, text-mining, and homology, respectively. STRING 11.0 was used to construct the interaction network.

Discussion

The literature review demonstrates the reports about gene pathways and mechanism of resistance to VCR in ALL cells of patients. However, most of them are B-lineage cells or a combination of B- and T- cells (4-6). Then, the effect of VCR on gene profile of T-ALL cells has not been specifically evaluated until now. Here, we investigated human cancer drug target gene expression changes in Jurkat cells treated by VCR. The mRNA level of 84 genes in seventeen pathways was compared between treated cells and untreated cells (negative control). The pathways included apoptosis, PI3-kinase and phosphatase, growth factors and receptors, drug metabolism, G protein signaling, hormone receptors, heat shock proteins, receptor tyrosine kinase signaling, cathepsins, cell cycle, topoisomerase type II, transcription factors, protein kinases, RAS signaling, histone deacetylases, poly ADPribose polymerase, structural protein. The gene expression consideration revealed that VCR induced cell cycle inhibition by decreasing mRNA levels of CDK1, CDK2, CDK5, CDK8 and CDK9. Cyclin-dependent kinases (CDKs) are serine-threonine protein kinases with a direct role in the regulation of the mitosis cell cycle and an indirect role in meiosis, transcription, and DNA repair (13). VCR treatment, also, caused down-regulation of MDM2 and MDM4. MDM2/MDM4 complex decreases the protein level of p53 (a tumor suppressor) (14). TERT was another gene in the cell cycle pathway and was up regulated in this study. TERT is a part of telomerase that catalyzes increasing and stabilizing telomere length. The activity of telomerase results in immortalizing the cells and is critical in carcinogenesis. Previous studies showed telomerase activity was higher in children with acute leukemia in comparison to healthy persons (15). So TERT was one of evaluated genes that resisted against cytotoxic effect of VCR in Jurkat cells.

The expression of CTSD, CTSL and CTSS was decreased under the influence of VCR. The intensity of expression reduction was

higher for CTSL and CTSS genes. Cathepsins (CTS) play an important role in tumor progression. Overexpression of these proteins is correlated with cancers (16).

Apoptosis effects of VCR on Jurkat cells led to down-regulating TOP2A, TOP2B, HSP90AA1, HSP90B1 and transcription factors (ATF2, HIF1A). genes Topoisomerases are necessary for many DNA functions during normal cell growth and, also, are trigger in various physiologic and nonphysiologic stresses because of their role in DNA breaking and reconnecting (17). ATF2, HIF1 and HSP90 are anti-apoptotic proteins and overexpressed in cell stress conditions (18-20). As shown in Fig. 2, both of TOP2A and TOP2B have direct interaction with CDK1. HSP90AA1 is a key protein in the gene network activated by VCR and has the most association with other proteins.

The expression level of HDAC2 and HDAC3 in treated cells was 9.53 and 2.23 times lower than untreated cells, respectively. HDAC4 and HDAC8 were up regulated, but they can reduce apoptotic effect of VCR because in their expression level changes are very minor. Histone deacetylases (HDACs) result in chromatin condensation by histone deacetylation and facilitate gene expression. Several studies have shown the anti-leukemic activity of HDAC inhibitors and the potential role of HDACs in treatment of leukemia (21). was indicated VCR decreased gene transcription in ALL cells, so that the association between HDAC3 and ATF2 (a transcription factor) was shown in Fig. 2.

Down-regulation of the PI3- kinase and phosphatase genes (PIK3C2A, PIK3C3 and PIK3CA) and growth factors and receptor genes (EGFR, ERBB3, ERBB4, FLT1, IGF1R, KDR and KIT) was a response to apoptosis signals of VCR. The member of PIK3C group play several roles in angiogenesis, cell growth and tumor formation (through activity in mTOR signaling pathway) and cell cycle (22, 23). PIK3CA had an interaction with MDM2 in Fig. 2. Bartram et al

described that IGF1R inhibition was effective in decreasing proliferation of T-ALL cells (Jurkat and Molt-4) resistant to VCR and ASP (24). Our results confirmed IGF1R, as a growth receptor, was one of the target genes in Jurkat cells treated by VCR. KIT is a tyrosine kinase receptor and leukemia is the most cancer related to KIT gene mutation (25).

Overexpression of glutathione-s-transferase p1 (GSTP1) is related to resistance to anticancer drugs (26). Expression of ABCC1, also, increases in many human cancer cell lines with multi-drug resistance (27). Then VCR increased the sensitivity of Jurkat cells to drug effect by down-regulating GSTP1 and ABCC1 genes.

According signaling to our results, pathways and hormone receptors were affected in T-ALL cells by VCR treatment. So that, RHOA (G protein signaling), GRB2 (receptor tyrosine kinase signaling) and PGR (a hormone receptor) were downregulated. All the genes in protein kinases pathway were activated by VCR induction that the most of them enhanced cell death. AURKA, AURKB **AURKC** are three members and serine/threonine kinase family and regulators of mitosis and meiosis. Overexpression of AURKA and AURKB was detected in solid tumors and ALL and acute myeloid leukemia (AML) (28). In this research, the expression level of PLK genes was decreased in Jurkat cells. PLK1 overexpressed in various cancers and high expression of PLK4 is related to many cases of metastatic cancers. PLK2 is a tumor suppressor but PLK3 has a low expression in many neoplastic tissues, and it seems PLK3 is a pro-apoptotic protein (29). High expression of PRKC was shown in a study by Lei et al. They demonstrated overexpression of Rack1 (a receptor of PKC) resulted in raising expression level of PKCα in resistant T-ALL cell lines (Jurkat and CCRF-CEM) to VCR (30). Down- regulation of

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PRKCD and PRKCE genes had a positive effect on Jurkat cell apoptotic events in our study.

Decreased mRNA level of KRAS, PARP1 and TNKS genes were other feedbacks of apoptotic events in Jurkat cells due to treatment by VCR.

Some evaluated genes showed resistance to VCR apoptosis induction by increasing expression level, including BIRC5, MTOR, ERBB2, FLT4, IGF1, IGF2, ESR1, NRAS, PARP2. But also, decreasing mRNA level of some genes was resulted in cell survival and resistance to cell death, including TXNRD1, IRF5 and PARP4. From up-regulated genes, ESR1 is the most important resistant gene and interacts with four up-regulated genes of other pathways including TERT, HDAC4, MTOR and IGF1 which had a negative effect on VCR apoptotic process (Fig. 2). ESR1 stimulates the proliferation of cancer cells (31).

In conclusions, in most of evaluated pathways, there were genes with expression changes resulted from VCR apoptotic signals, although some genes were without changes and some genes were resistant to VCR treatment. In this study exactly VCR target genes in T-ALL cells were determined that can be used as biomarkers for ALL therapy and future studies about new anti-leukemic agents. Also, these results can be used to select appropriate drugs to synergize the effect of VCR to improve ALL treatment.

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