Research Article

Ceren Sucularli*, Ugur Toprak and Melda Arslantas

Gene expression data analysis for characterizing shared and type specific mechanisms of HCC and B-CLL

[HCC ve B-CLL'in ortak ve tipe özgü mekanizmalarının karakterizasyonu için gen ifadesi analizleri]

https://doi.org/10.1515/tjb-2018-0039
Received February 7, 2018; accepted June 19, 2018; previously published online September 10, 2018

Abstract

Background: Comparing gene expression profiles using gene expression datasets of different types of tumors is frequently used to identify molecular mechanisms of cancer. This study aimed to find shared and type specific gene expression profiles of hepatocellular carcinoma (HCC) and B-cell chronic lymphocytic leukemia (B-CLL).
Material and methods: Gene expression microarrays for HCC and B-CLL and RNA-sequencing expression data for liver HCC and lymphoid neoplasm diffuse large B-cell lymphoma (DLBC) were analyzed and differentially expressed probe sets or genes for each cancer type were detected. Probe sets and genes that were shared or specifically expressed in both cancer types were identified. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) terms for Biological Process (BP) annotations were performed.
Results: According to our analysis shared upregulated genes were mainly annotated in cell cycle processes. Some of the genes that changed only in HCC were annotated in cell cycle and metabolic processes, and some of the genes, altered only in B-CLL, were annotated in immune response and hemopoiesis.
Conclusion: These results contribute to cancer research that aim to find the conserved gene expression profiles in different cancer types and widen the knowledge of HCC and B-CLL specific mechanisms.

Keywords: B-cell chronic lymphocytic leukemia; Hepatocellular carcinoma; Re-analysis of public data; Gene expression; Bioinformatics; Microarray.

Öz

Amaç: Farklı tümörlere ait gen ifadesi veri setlerini karşılaştırmak, kanserlerin moleküler mekanizmalarını açığa çıkarmak için kullanılmaktadır. Bu çalışma hepatosellüler karsinom (HCC) ve B-hücreli kronik lenfositik lösemi (B-CLL) arasında paylaşılan veya tipe özgü olan gen ifade profillerini bulmayı amaçlamaktadır.
Gereç ve Yöntem: HCC ve B-CLL için gen ifade mikrodizileri ve karaciğer hepatosellüler karsinom ve diffüz büyük B hücreli lenfoma için RNA-seq ifade verileri analiz edilmiş ve iki kanser tipi için ifadesi farklı değişen prob setler ya da genler saptanmıştır. HCC ve B-CLL arasında ortak olan veya özgül olarak ifade olan prob setler ya da genler belirlenmiştir. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway ve Gen Ontolojisi (GO) Biyolojik Proses (BP) terimleri açıklamaları gerçekleştirilmiştir.
Bulgular: Analizlerimize göre ifadesi artan genlerden ortak olanlar çoğunlukla hücre döngüsü süreçlerinde rol alıyor olarak saptanmıştır. İfadesi sadece HCC'de değişen genlerin bir kısmı hücre döngüsü ve metabolik süreçlerde

***Corresponding author: Dr. Ceren Sucularli,** Department of Bioinformatics, Institute of Health Sciences, Hacettepe University, Ankara 06100, Turkey, Phone: +90312 3053038, Fax: +90312 3051459, e-mail: ceren.sucularli@hacettepe.edu.tr. https://orcid.org/0000-0003-0627-7900
Ugur Toprak: Department of Biostatistics, Faculty of Medicine, Ankara University, Ankara 06100, Turkey, e-mail: toprakugur@ymail.com
Melda Arslantas: Department of Bioinformatics, Institute of Health Sciences, Hacettepe University, Ankara 06100, Turkey, e-mail: meldaarslantas@hotmail.com

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

ve ifadesi sadece B-CLL'de değişen genlerin bir kısmı immün cevap ve hemopoiez terimlerinde rol alıyor olarak bulunmuştur.

Sonuç: Bu sonuçlar, farklı kanser tiplerine ait korunmuş gen ifade profillerini bulmayı amaçlayan kanser araştırmalarına katkı sağlayacak ve HCC ve B-CLL'ye özgü mekanizmalara ait bilgimizi genişletecektir.

Anahtar Kelimeler: B-hücreli kronik lenfositik lösemi; Hepatosellüler karsinom; Halka açık verilerin yeniden analizi; Gen ifadesi; Biyoinformatik; Mikrodizi.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the ninth in women, and the second most common reason of cancer mortality worldwide [1]. Geographic location, gender, age, ethnicity, hepatitis B viruses (HBV) and hepatitis C viruses (HCV) infections, obesity and diabetes mellitus have been correlated with HCC. In addition, rare inherited disorders, such as glycogen storage disease, alpha-1-antitrypsin deficiency, metal storage disease and chronic cholestatic syndromes increase the risk of HCC [2]. At the molecular level, activation of several Wnt, MAPK signaling pathway members, inactivation of p53 and Rb signaling pathways have been reported in HCC [3].

B-cell chronic lymphocytic leukemia (B-CLL), which is also known as chronic lymphoid leukemia (CLL), is the most common type of human leukemia [4]. Gender, age, ethnicity and family history of B-CLL or lymphoid malignancies are related with the disease. At the clinical course, B-CLL shows heterogeneity, the progress may occur either slowly, in which patients live for a long time without treatment, or aggressively [5]. There are several steps during B-CLL development, such as increase in lymphocyte number, aberrant gene expression and immunophenotypic changes. Germline mutations in the immunoglobulin heavy-chain variable genes, mutations and deregulation of p53 signaling pathway genes have been correlated with B-CLL formation [5].

Although, B-CLL arise from a different origin, hepatitis infections and invasion of liver by B-cells have been reported in B-cell malignancies [6]. Interestingly, the occurrence of B-cell malignancies, such as B-CLL and non-Hodgkin's lymphoma, and HCC has also been reported in patients without cirrhosis or hepatitis infection [7]. Although the occurrence of B-cell malignancies and HCC has been reported and some common risk factors have been described, the molecular mechanisms and bases to associate or to differentiate these cancers remain unsolved.

Integrating microarray studies and re-evaluating data sets have been used to reveal the molecular and regulatory mechanisms of different cancers, such as head and neck squamous-cell carcinoma, colorectal cancer (CRC), lung, ovary, gastric, liver and kidney [8–10]. Recently, new candidate genes were identified and proposed as potential targets for CRC diagnosis and treatment, by re-analyzing a single series, GSE4107 [8].

Comparing gene expression data from different cancer types may facilitate us to decipher the core mechanisms involved in the cancer formation or progression. In this study, we aimed to find the shared and type specific gene sets for HCC and B-CLL to expand our understanding in tumor formation and development, and to detect potential targets for further studies. We searched public databases, GEO and ArrayExpress, for HCC and B-CLL gene expression studies. We identified and compared differentially expressed genes for HCC and B-CLL from two distinct microarray data sets. In addition to microarray datasets, we compared the RNA-sequencing expression datasets for liver hepatocellular carcinoma (LIHC) and DLBC and identified differentially expressed genes between two cancers. The KEGG pathway and GO terms for BP annotations of common and specific genes showed that cell cycle was altered in both cancer types. Our results also revealed induced or repressed KEGG pathway and BP terms in a cancer type specific manner. In addition, after microarray analysis, we detected that different genes of KEGG pathway "Pathways in cancer" were affected in both cancers. Results of this study enhanced our understanding and knowledge about shared and type specific mechanisms of HCC and B-CLL.

Materials and methods

Data retrieval

Data sets for HCC and B-CLL were searched in Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/, [11, 12]) and ArrayExpress (https://www.ebi.ac.uk/arrayexpress/, [13]) databases. One record from HCC (GSE40367, [14]) and one from B-CLL (GSE26725, [15]) were selected and downloaded from GEO. Both records were on GPL570 platform and had patient and control samples. CEL files from GSE40367 (10 HCC without metastasis primary tumor samples and five normal liver of hemangioma samples) and from GSE26725 (12 peripheral blood samples of B-CLL

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

patients and five peripheral blood samples of healthy donors) were used for the study (Table 1).

Microarray analysis

CEL files were Robust Multichip Average (RMA, [16]) normalized with Affymetrix Expression Console version 1.4.1.46 (http://www.affymetrix.com). After normalization, significantly changed probe sets and their annotated genes were determined by using Affymetrix Transcriptome Analysis Console version 3.0.0.466 (http://www.affymetrix.com). One-way between-subject ANOVA (unpaired) was used to compare the intensities of patient samples to intensities of normal/control samples. The probe sets with ANOVA p-value <0.05 and fold change (linear) <–2 or fold change (linear) >2 were considered as differentially expressed.

Identification of shared and HCC, B-CLL only probe sets

The significantly upregulated/downregulated probe sets of HCC (HCC vs. control) were compared to significantly upregulated/downregulated probe sets of B-CLL (B-CLL vs. control) with Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html, [17]) to find the shared and type specific probe sets between HCC and B-CLL.

Table 1: Data sets that are used for this study.

HCC (GSE40367, [14])	B-CLL (GSE26725, [15])
Control	
GSM992287	GSM658010
GSM992288	GSM658011
GSM992289	GSM658012
GSM992290	GSM658013
GSM992291	GSM658014
Patient	
GSM992292	GSM658015
GSM992293	GSM658016
GSM992294	GSM658017
GSM992295	GSM658018
GSM992296	GSM658019
GSM992297	GSM658020
GSM992298	GSM658021
GSM992299	GSM658022
GSM992300	GSM658023
GSM992301	GSM658024
	GSM658025
	GSM658026

HCC, Hepatocellular carcinoma; B-CLL, B-cell chronic lymphocytic leukemia; GSM, Gene Expression Omnibus Sample.

GO BP and KEGG pathway analyses

The upregulated and downregulated probe sets that were shared between HCC and B-CLL, and the probe sets that were specific to HCC or B-CLL were consecutively uploaded to The Database for Annotation, Visualization and Integrated Discovery version 6.7 (DAVID, https://david.ncifcrf.gov/, [18, 19]), which has extensive using areas and enables to find out biological meaning of gene lists. The significant GO BP terms and KEGG pathways were selected according to p-value. $p < 0.05$ was considered as significant.

Cluster analysis

In order to prepare heat-maps of 57 upregulated and 69 downregulated probe sets that were shared between HCC and B-CLL, probe sets and arrays were hierarchically clustered according to Euclidian distance similarity metric and average linkage clustering with Cluster 3.0 [20, 21] and visualized with Java Treeview version 1.1.6r4 [22] for each cancer type.

The RNA sequencing expression data analyses for LIHC and DLBC

The RNA sequencing expression data of (LIHC; 369 tumor samples and 50 normal samples from TCGA, The Cancer Genome Atlas, and 110 normal liver samples from GTEx, Genotype-Tissue Expression) and (DLBC; 47 tumor samples from TCGA and 337 normal blood samples from GTEx) were analyzed by GEPIA (http://gepia.cancer-pku.cn/, [23]). For identification of differentially expressed genes ANOVA was performed, the Benjamini and Hochberg FDR method was applied to adjust the p-value to obtain the multiple testing adjusted q-value. The genes with $|\log2FC| > 2$ and q value < 0.05 were considered as differentially expressed.

The significantly upregulated/downregulated genes of LIHC and DLBC were compared with GeneVenn (http://genevenn.sourceforge.net/, [24]) and upregulated and downregulated genes that were shared between LIHC and DLBC or that were specific to LIHC and DLBC were analyzed for GO BP and KEGG pathway terms with DAVID 6.7 [18, 19]. $p < 0.05$ was considered as significant.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

Comparison of significant GO BP terms for Microarray and RNA-seq differentially expressed genes

Microarray and RNA-seq GO BP terms were compared and significant shared terms ($p < 0.05$) were analyzed for an association with two-tailed Fisher's exact test by using GraphPad QuickCalcs, Accessed May 2018 (https://www.graphpad.com/quickcalcs/contingency1/). $p < 0.05$ was considered as significant.

Results

Identification of differentially expressed microarray probe sets in HCC

In attempt to find shared and cancer type specific gene sets, we first analyzed HCC and B-CLL microarray data sets, separately. We analyzed tumor endothelium from HCC tumor tissue of HCC patients without metastasis primary tumor ($n = 10$) and tumor endothelium from normal liver tissue of hemangioma patients ($n = 5$) sample data from the HCC microarray data set, GSE40367 [14]. We identified 2339 probe sets, 1021 upregulated and 1318 downregulated, which were significantly changed [ANOVA p-value <0.05 and fold change (linear) <–2 or fold change (linear) >2] in HCC patient primary tumors compared with the controls.

Identification of differentially expressed microarray probe sets in B-CLL

In order to identify gene sets having differential expression in B-CLL, we analyzed CEL files of peripheral blood samples from B-CLL patients ($n = 12$) and healthy controls ($n = 5$) from GSE26725 microarray data set [15]. According to our comparison, 1976 probe sets were differentially expressed [ANOVA p-value <0.05 and fold change (linear) <–2 or fold change (linear) >2] in B-CLL patients. Of the 1976 probe sets, 930 probe sets were found to be upregulated, while 1046 were downregulated.

Identification of shared and cancer type specific microarray probe sets

In this study, we aimed to find shared and HCC and B-CLL specific mechanisms. Therefore, we compared the upregulated and downregulated probe sets of HCC to up and downregulated probe sets of B-CLL, respectively. Among the upregulated probe sets in both cancers, 57 probe sets were found to be shared between HCC and B-CLL (Figure 1). In the downregulated probe set list, 69 probe sets were common in both cancers (Figure 1).

In addition to shared upregulated and downregulated probe sets in HCC and B-CLL, we detected the probe sets that were upregulated or downregulated in a type specific manner (Figure 1). According to our analysis most of the upregulated or downregulated probe sets were found to be specific to cancer types.

Functional annotation of shared microarray genes

In order to detect the pathways and biological processes (BPs) that were related to the carcinogenesis, we analyzed the shared upregulated and downregulated probe sets with DAVID functional annotation tool version 6.7 [18, 19]. The shared upregulated probe sets were significantly annotated into 12 BP terms and were not annotated

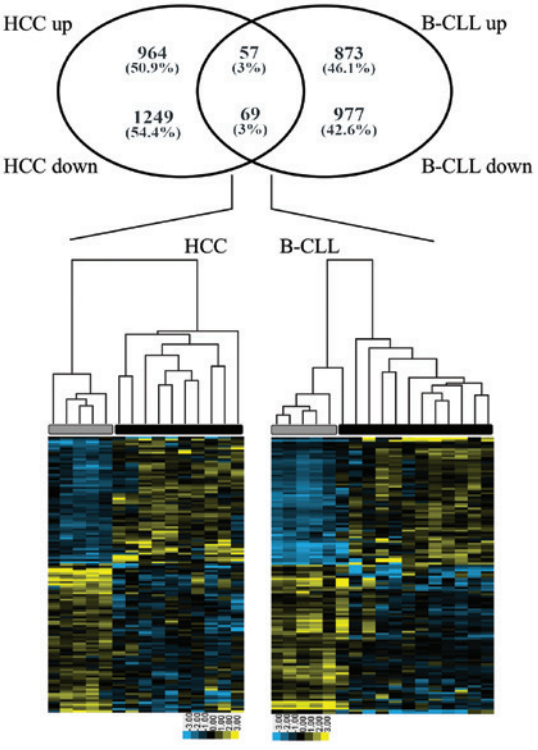


Figure 1: The Venn diagram of the shared and type specific significant microarray probe sets in HCC and B-CLL.
The heat maps of shared upregulated and downregulated probe sets in each cancer type.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

to any KEGG pathways, while the shared downregulated probe sets were annotated into 28 BP terms and one KEGG pathway term. The shared upregulated genes were significantly annotated in BP terms mainly related to mitotic cell cycle processes (Table 2). According to BP annotations, the shared downregulated genes were involved in positive regulation of macromolecule biosynthetic process and endocytosis related terms (Table 2). In addition, three of the downregulated probe sets were annotated to Hematopoietic cell lineage KEGG pathway ($p = 0.036$, data not shown).

Functional annotation of type specific microarray genes

According to functional annotation of HCC specific genes, the expression of genes that altered only in HCC functioned in cell cycle, DNA replication and metabolic processes (Tables 3 and 4), and several genes that differentially expressed only in B-CLL involved in immune response, regulation of apoptosis and hemopoiesis (Tables 5 and 6). Interestingly, KEGG pathway "Pathways in cancer" appeared as upregulated in both HCC and B-CLL specific KEGG terms (Tables 4 and 6). When we searched for the genes in this term, we recognized that different proteins of this pathway were upregulated in HCC and B-CLL (Figure 2 and Table S1).

Differentially expressed genes for LIHC and DLBC RNA-seq datasets

We analyzed RNA-seq expression data for liver hepatocellular carcinoma, LIHC, and DLBC, with their corresponding normal samples to identify the shared and type specific differentially expressed genes.

We identified 263 genes, 118 upregulated and 145 downregulated, significantly changed in LIHC ($|\log2FC| > 2$ and q value < 0.05).

When we analyzed DLBC, we identified 4536 genes, 4110 upregulated and 426 downregulated, that were significantly changed in DLBC ($|\log2FC| > 2$ and q value < 0.05).

In an attempt to find shared and type specific genes between LIHC and DLBC, we identified that 73 upregulated and eight downregulated genes were shared between LIHC and DLBC. Forty five upregulated and 137 downregulated genes were specifically changed in liver hepatocellular carcinoma, while 4037 upregulated and 418 downregulated genes were changed only in DLBC.

Table 2: The top 10 GO terms for BP of shared upregulated and downregulated genes between HCC and B-CLL from microarray analysis ($p < 0.05$).

Term	Description	Count	p-Value	Benjamini
Upregulated – BP				
GO:0000278	Mitotic cell cycle	7	4.56E-04	2.11E-01
GO:0022402	Cell cycle process	8	7.37E-04	1.75E-01
GO:0007052	Mitotic spindle organization	3	7.47E-04	1.22E-01
GO:0022403	Cell cycle phase	7	8.23E-04	1.02E-01
GO:0007049	Cell cycle	8	4.52E-03	3.76E-01
GO:0007051	Spindle organization	3	6.69E-03	4.41E-01
GO:0051301	Cell division	5	8.31E-03	4.62E-01
GO:0043933	Macromolecular complex subunit organization	7	1.18E-02	5.38E-01
GO:0000279	M phase	5	1.21E-02	5.04E-01
GO:0042026	Protein refolding	2	1.36E-02	5.09E-01
Downregulated – BP				
GO:0010557	Positive regulation of macromolecule biosynthetic process	9	9.22E-04	3.96E-01
GO:0031328	Positive regulation of cellular biosynthetic process	9	1.24E-03	2.89E-01
GO:0009891	Positive regulation of biosynthetic process	9	1.37E-03	2.21E-01
GO:0007167	Enzyme linked receptor protein signaling pathway	6	4.39E-03	4.52E-01
GO:0010604	Positive regulation of macromolecule metabolic process	9	5.02E-03	4.23E-01
GO:0006897	Endocytosis	5	5.11E-03	3.73E-01
GO:0010324	Membrane invagination	5	5.11E-03	3.73E-01
GO:0007169	Transmembrane receptor protein tyrosine kinase signaling pathway	5	5.45E-03	3.47E-01
GO:0016044	Membrane organization	6	6.90E-03	3.77E-01
GO:0010033	Response to organic substance	8	7.14E-03	3.53E-01

GO, Gene Ontology; BP, Biological Process; HCC, hepatocellular carcinoma; B-CLL, B-cell chronic lymphocytic leukemia; Count, number of genes involved in the term; p-value, modified Fisher exact p-value, EASE score; Benjamini, Benjamini-Hochberg corrected p-value.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

Table 3: The top 10 GO terms for BP of HCC specific upregulated and downregulated genes from microarray analysis ($p < 0.05$).

Term	Description	Count	p-Value	Benjamini
Upregulated – BP				
GO:0007049	Cell cycle	114	3.16E-30	7.87E-27
GO:0022403	Cell cycle phase	80	5.99E-29	7.46E-26
GO:0000279	M phase	69	4.11E-27	3.41E-24
GO:0022402	Cell cycle process	90	3.25E-26	2.02E-23
GO:0000278	Mitotic cell cycle	71	1.81E-25	9.02E-23
GO:0000280	Nuclear division	54	1.27E-24	5.25E-22
GO:0007067	Mitosis	54	1.27E-24	5.25E-22
GO:0048285	Organelle fission	55	1.37E-24	4.89E-22
GO:0000087	M phase of mitotic cell cycle	54	3.17E-24	9.87E-22
GO:0051301	Cell division	53	9.24E-18	2.56E-15
Downregulated – BP				
GO:0055114	Oxidation reduction	108	1.66E-32	4.50E-29
GO:0016054	Organic acid catabolic process	39	2.46E-23	3.34E-20
GO:0046395	Carboxylic acid catabolic process	39	2.46E-23	3.34E-20
GO:0006631	Fatty acid metabolic process	42	4.31E-16	4.02E-13
GO:0009310	Amine catabolic process	26	5.50E-15	3.77E-12
GO:0009063	Cellular amino acid catabolic process	24	1.80E-14	9.76E-12
GO:0019439	Aromatic compound catabolic process	14	2.51E-13	1.14E-10
GO:0046394	Carboxylic acid biosynthetic process	32	4.32E-12	1.68E-09
GO:0016053	Organic acid biosynthetic process	32	4.32E-12	1.68E-09
GO:0051186	Cofactor metabolic process	34	1.01E-10	3.41E-08

GO, Gene Ontology; BP, Biological Process; HCC, hepatocellular carcinoma; Count, number of genes involved in the term; p-value, modified Fisher exact p-value, EASE score; Benjamini, Benjamini-Hochberg corrected p-value.

Table 4: The top 10 KEGG pathway terms of HCC specific upregulated and downregulated genes from microarray analysis ($p < 0.05$).

Term	Description	Count	p-Value	Benjamini
Upregulated – KEGG				
hsa04110	Cell cycle	31	4.61E-14	7.14E-12
hsa03030	DNA replication	9	2.22E-04	1.71E-02
hsa04115	p53 signaling pathway	12	3.11E-04	1.59E-02
hsa05200	Pathways in cancer	29	1.60E-03	6.02E-02
hsa05222	Small cell lung cancer	12	1.92E-03	5.79E-02
hsa04114	Oocyte meiosis	13	5.69E-03	1.37E-01
hsa04914	Progesterone-mediated oocyte maturation	11	7.23E-03	1.48E-01
hsa04666	Fc gamma R-mediated phagocytosis	11	1.42E-02	2.42E-01
hsa04120	Ubiquitin mediated proteolysis	13	2.91E-02	3.98E-01
hsa03040	Spliceosome	12	3.66E-02	4.39E-01
Downregulated – KEGG				
hsa00982	Drug metabolism	26	1.80E-14	3.11E-12
hsa00830	Retinol metabolism	24	5.07E-14	4.35E-12
hsa00071	Fatty acid metabolism	20	7.63E-13	4.37E-11
hsa00280	Valine, leucine and isoleucine degradation	20	6.42E-12	2.76E-10
hsa00380	Tryptophan metabolism	19	9.94E-12	3.42E-10
hsa03320	PPAR signaling pathway	22	1.17E-09	3.35E-08
hsa00980	Metabolism of xenobiotics by cytochrome P450	19	2.40E-08	5.90E-07
hsa00260	Glycine, serine and threonine metabolism	13	2.40E-07	5.16E-06
hsa00350	Tyrosine metabolism	15	4.01E-07	7.66E-06
hsa00650	Butanoate metabolism	13	7.79E-07	1.34E-05

KEGG, Kyoto Encyclopedia of Genes and Genomes; HCC, hepatocellular carcinoma; Count, number of genes involved in the term; p-value, modified Fisher exact p-value, EASE score; Benjamini, Benjamini-Hochberg corrected p-value.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

Table 5: The top 10 GO terms for BP of B-CLL specific upregulated and downregulated genes from microarray analysis ($p < 0.05$).

Term	Description	Count	p-Value	Benjamini
Upregulated – BP				
GO:0030097	Hemopoiesis	20	9.02E-05	1.89E-01
GO:0048534	Hemopoietic or lymphoid organ development	20	3.16E-04	3.07E-01
GO:0046907	Intracellular transport	37	3.45E-04	2.34E-01
GO:0015671	Oxygen transport	5	4.57E-04	2.33E-01
GO:0010629	Negative regulation of gene expression	30	5.93E-04	2.41E-01
GO:0002520	Immune system development	20	6.61E-04	2.26E-01
GO:0001932	Regulation of protein amino acid phosphorylation	15	7.09E-04	2.09E-01
GO:0050863	Regulation of T cell activation	12	7.63E-04	1.98E-01
GO:0006468	Protein amino acid phosphorylation	36	8.82E-04	2.03E-01
GO:0042981	Regulation of apoptosis	41	1.09E-03	2.24E-01
Downregulated – BP				
GO:0006955	Immune response	59	1.12E-10	2.89E-07
GO:0007204	Elevation of cytosolic calcium ion concentration	18	1.59E-07	2.05E-04
GO:0051480	Cytosolic calcium ion homeostasis	18	4.49E-07	3.86E-04
GO:0001775	Cell activation	28	1.62E-06	1.04E-03
GO:0002684	Positive regulation of immune system process	25	1.83E-06	9.43E-04
GO:0046649	Lymphocyte activation	22	3.95E-06	1.69E-03
GO:0045321	Leukocyte activation	24	8.11E-06	2.98E-03
GO:0019725	Cellular homeostasis	36	8.80E-06	2.83E-03
GO:0007050	Cell cycle arrest	15	9.32E-06	2.67E-03
GO:0010557	Positive regulation of macromolecule biosynthetic process	45	1.05E-05	2.71E-03

GO, Gene Ontology; BP, Biological Process; B-CLL, B-cell chronic lymphocytic leukemia; Count, number of genes involved in the term; p-value, modified Fisher exact p-value, EASE score; Benjamini, Benjamini-Hochberg corrected p-value.

Table 6: The top 10 KEGG pathway terms of B-CLL upregulated and downregulated genes from microarray analysis ($p < 0.05$).

Term	Description	Count	p-Value	Benjamini
Upregulated – KEGG				
hsa04722	Neurotrophin signaling pathway	12	1.84E-03	2.08E-01
hsa05215	Prostate cancer	10	1.94E-03	1.16E-01
hsa04916	Melanogenesis	10	4.02E-03	1.57E-01
hsa00600	Sphingolipid metabolism	6	7.40E-03	2.10E-01
hsa04912	GnRH signaling pathway	9	1.23E-02	2.71E-01
hsa05200	Pathways in cancer	19	1.58E-02	2.87E-01
hsa03040	Spliceosome	10	1.85E-02	2.87E-01
hsa04920	Adipocytokine signaling pathway	7	1.92E-02	2.64E-01
hsa04670	Leukocyte transendothelial migration	9	3.37E-02	3.84E-01
hsa04320	Dorso-ventral axis formation	4	4.37E-02	4.33E-01
Downregulated – KEGG				
hsa04640	Hematopoietic cell lineage	16	9.72E-07	1.35E-04
hsa04672	Intestinal immune network for IgA production	11	1.59E-05	1.10E-03
hsa05020	Prion diseases	8	3.55E-04	1.63E-02
hsa04062	Chemokine signaling pathway	18	1.04E-03	3.54E-02
hsa04612	Antigen processing and presentation	9	1.61E-02	3.63E-01
hsa05332	Graft-versus-host disease	6	1.77E-02	3.39E-01
hsa04940	Type I diabetes mellitus	6	2.38E-02	3.80E-01
hsa05220	Chronic myeloid leukemia	8	2.78E-02	3.87E-01
hsa04514	Cell adhesion molecules (CAMs)	11	3.52E-02	4.25E-01
hsa04670	Leukocyte transendothelial migration	10	4.29E-02	4.56E-01

KEGG, Kyoto Encyclopedia of Genes and Genomes; B-CLL, B-cell chronic lymphocytic leukemia; Count, number of genes involved in the term; p-value, modified Fisher exact p-value, EASE score; Benjamini, Benjamini-Hochberg corrected p-value.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

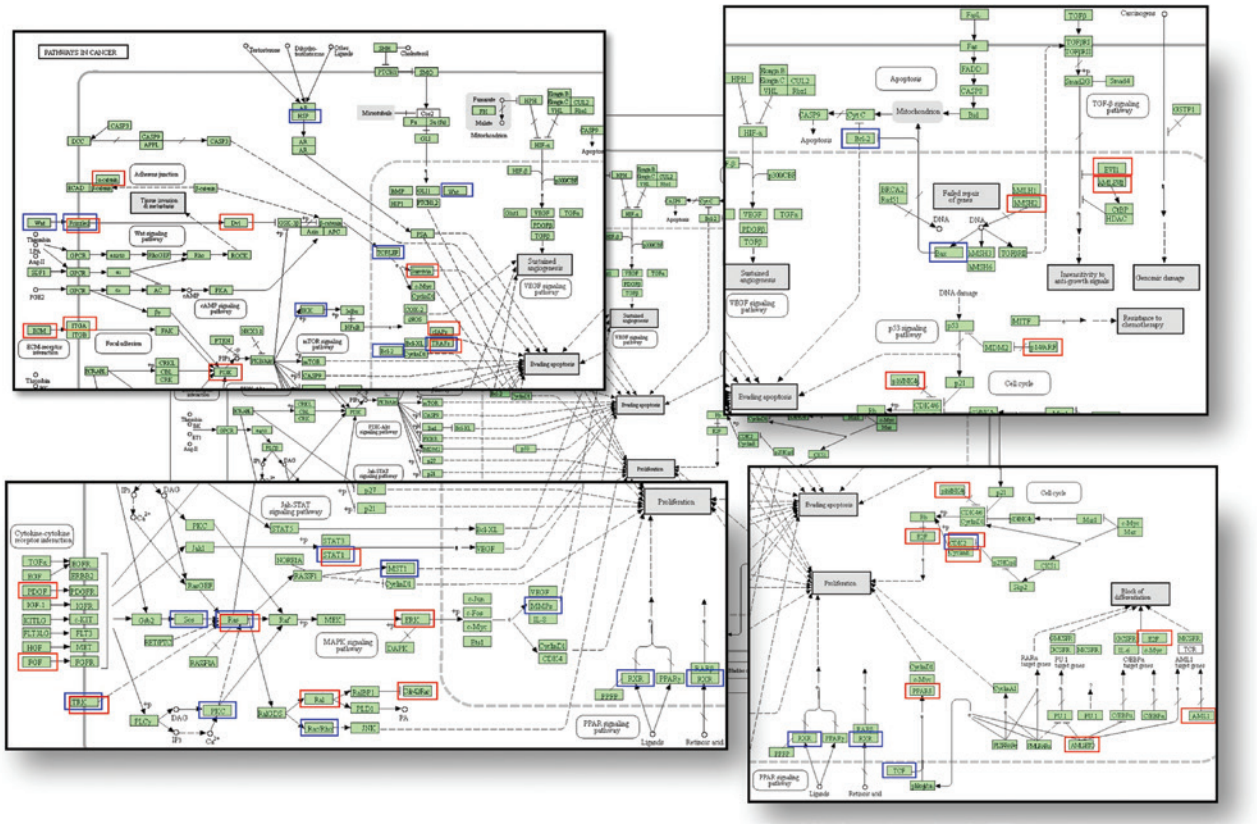


Figure 2: The upregulated probe sets of HCC (red boxes) and B-CLL (blue boxes) in "Pathways in cancer" term of KEGG pathway.

Functional annotation of shared or LIHC and DLBC specific genes

The shared and type specific upregulated and downregulated genes between LIHC and DLBC were annotated to GO BP and KEGG pathway terms.

According our analysis, shared upregulated genes were significantly annotated to cell cycle related GO BP terms, while shared downregulated genes were significantly annotated to oxygen and gas transport GO BP terms (Table 7).

The expression of several genes that changed only in LIHC functioned in metabolic processes, such as retinol metabolism and drug metabolism KEGG pathway terms, and homeostasis related GO BP terms (Table 8 and data not shown), and several genes that differentially expressed

Table 7: The top five GO terms for BP of LIHC and DLBC shared upregulated and downregulated genes ($p < 0.05$).

Term	Description	Count	p-Value	Benjamini
Upregulated – BP				
GO:0000278	Mitotic cell cycle	19	3.78E-16	2.72E-13
GO:0022403	Cell cycle phase	19	2.79E-15	1.13E-12
GO:0000279	M phase	16	4.68E-13	1.27E-10
GO:0022402	Cell cycle process	19	5.94E-13	1.21E-10
GO:0007049	Cell cycle	21	1.08E-12	1.76E-10
Downregulated – BP				
GO:0015671	Oxygen transport	2	3.84E-03	4.21E-01
GO:0015669	Gas transport	2	5.31E-03	3.15E-01

GO, Gene Ontology; BP, Biological Process; LIHC, liver hepatocellular carcinoma dataset; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma dataset; Count, number of genes involved in the term; p-value, modified Fisher exact p-value, EASE score; Benjamini, Benjamini-Hochberg corrected p-value.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

Table 8: The top five GO terms for BP of LIHC specific and DLBC specific upregulated and downregulated genes ($p < 0.05$).

		LIHC specific terms				DLBC specific terms			
Term	**Description**	**Count**	**p-Value**	**Benjamini**	**Term**	**Description**	**Count**	**p-Value**	**Benjamini**
Upregulated – BP					Upregulated – BP				
GO:0008285	Negative regulation of cell proliferation	4	1.53E-02	9.99E-01	GO:0006412	Translation	230	4.28E-87	1.82E-83
GO:0009617	Response to bacterium	3	3.25E-02	9.99E-01	GO:0006396	RNA processing	290	1.35E-69	2.87E-66
GO:0009611	Response to wounding	4	4.15E-02	9.98E-01	GO:0022613	Ribonucleoprotein complex biogenesis	131	5.81E-53	8.24E-50
GO:0008637	Apoptotic mitochondrial changes	2	4.49E-02	9.92E-01	GO:0034660	ncRNA metabolic process	144	9.49E-46	1.01E-42
					GO:0006414	Translational elongation	84	9.94E-42	8.46E-39
Downregulated – BP					Downregulated – BP				
GO:0048878	Chemical homeostasis	18	1.52E-08	1.74E-05	GO:0006952	Defense response	58	6.43E-23	1.05E-19
GO:0002526	Acute inflammatory response	8	2.62E-06	1.51E-03	GO:0006955	İmmune response	57	9.09E-20	7.45E-17
GO:0042592	Homeostatic process	18	3.43E-06	1.32E-03	GO:0006954	İnflammatory response	31	2.91E-12	1.59E-09
GO:0030003	Cellular cation homeostasis	11	4.15E-06	1.19E-03	GO:0009611	Response to wounding	39	7.67E-12	3.14E-09
GO:0055082	Cellular chemical homeostasis	13	4.20E-06	9.67E-04	GO:0042742	Defense response to bacterium	18	8.46E-11	2.77E-08

GO, Gene Ontology; BP, Biological Process; LIHC, liver hepatocellular carcinoma dataset; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma dataset; Count, number of genes involved in the term; p-value, modified Fisher exact p-value, EASE score; Benjamini, Benjamini-Hochberg corrected p-value.

only in DLBC involved in immune response, RNA processing and hematopoietic cell lineage GO BP and KEGG pathway terms (Table 8 and data not shown).

Association of GO BP terms from Microarray and RNA-seq analyses

In order to detect if there is a significant correlation for the GO BP terms that we obtained from the microarray and RNA-seq expression data analysis, we compared the shared and type specific terms from microarray analysis with their corresponding matches from RNA-seq dataset analysis. According to our results, there is a significant correlation between microarray and RNA-seq expression data analyses for the GO BP annotations of HCC and LIHC specific ($p = 0.0308$) and B-CLL and DLBC specific genes ($p = 0.0013$).

Since we could not detect any significant KEGG pathway term for shared downregulated genes and LIHC specific upregulated genes that we obtained from RNA-sequence expression data analysis and shared upregulated genes from microarray analysis, we could not compare the KEGG terms between microarray and RNA-sequence expression data.

Discussion

Altered gene expression or activating/inactivating mutations of common genes have been characterized in cancers originated from different tissues [25, 26]. Therefore, analyzing different cancer types to identify common genes or pathways that involve in cancer formation or progression might be an informative approach in cancer research. On the other hand, origin of the tissue influences the gene expression profile of the tumor cells and also cancers from different origins carry different gene expression signatures [25].

In order to identify conserved gene signatures and type specific gene expression in HCC and B-CLL we compared microarray gene expression data of HCC and B-CLL and RNA-sequencing expression data of LIHC and DLBC.

Our microarray analysis showed that expression of several genes involved in cell cycle were upregulated, while expression of the genes in endocytosis and positive regulation of biosynthetic processes were decreased in both cancers (Table 2). We also detected the gene expression alterations that were specific to each cancer type. According to our analysis, cell cycle related genes were upregulated in HCC (Tables 3 and 4), which might

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

be consistent with the increased proliferation of cancer cells. The genes coding for several metabolic pathways (Tables 3 and 4) were downregulated in HCC, which might reflect the disruption of the hepatic cell functions in liver carcinogenesis. Expression of several genes involved in immune response and hemopoiesis were significantly changed in B-CLL (Tables 5 and 6). Expression of different genes involved in "Pathways in cancer" term was upregulated in HCC and B-CLL (Figure 2 and Table S1), which results in appearance of this term in the upregulated KEGG term of both cancer types (Tables 4 and 6).

According to our RNA-sequencing expression data analysis, several shared genes between LIHC and DLBC datasets were annotated to cell cycle and related terms as in microarray analysis (Tables 2 and 7). Only two of the eight shared downregulated genes were significantly annotated to GO BP terms in our analysis, which were oxygen and gas transport terms (Table 7). When we analyzed LIHC specific upregulated and downregulated genes, we observed the downregulation of the genes involved in drug and retinol metabolism and metabolism of xenobiotics by cytochrome P450 KEGG pathway terms ($p < 0.05$, Benjamini < 0.05, data not shown), as we observed in microarray analysis (Table 4). According to DLBC specific GO BP and KEGG pathway analysis, many genes in RNA processing and translation were upregulated and the genes in hematopoietic cell lineage KEGG pathway term (data not shown), and immune response and related BP terms were downregulated in DLBC (Table 8).

The upregulated genes that were shared between HCC and B-CLL and also between LIHC and DLBC were mostly annotated to cell cycle related BP terms in our microarray and RNA-sequencing expression data analysis. Upregulation of cell cycle genes have been frequently associated with cancer types, such as ovarian cancer [27] and HCC [28]. In our microarray analysis, we observed upregulation of pim-1 proto-oncogene, serine/threonine kinase (*PIM1*), protein regulator of cytokinesis 1 (*PRC1*), branched-chain-amino-acid aminotransferase (*BCAT1*), double-strand-break repair protein rad21 homolog (*RAD21*), structural maintenance of chromosomes protein 3 (*SMC3*), kinetochore-associated protein 1 (*KNTC1*) and also cytoplasmic dynein 1 heavy chain 1 (*DYNC1H1*) in both B-CLL and HCC. According to our RNA-sequencing expression data analysis, only *PRC1* was upregulated in both LIHC and DLBC, while *RAD21*, *KNTC1, SMC3, DYNC1H1* and *BCAT1* were upregulated only in DLBC. Recently, *BCAT1*, which is essential for the catabolism of branched-chain amino acids; (BCAAs) valine, leucine and isoleucine, was proposed as a target gene for both HCC and lymphomagenesis [29]. BCAA catabolism and BCAT1 emerged as a target in cancer therapy; suppression of BCAA catabolism by inhibiting BCAT1 caused a decrease in cell proliferation in glioma cells by reducing energy production and macromolecule synthesis of tumor cells [30].

We observed upregulation of the genes involved in cell cycle and mitosis in HCC and LIHC (Tables 3, 4 and 7), consistent with the increased proliferation of hepatocytes after carcinogenesis. In our analysis, we observed downregulation of the genes involved in retinol and drug metabolism only in HCC and LIHC (Table 4 and data not shown). Previous studies showed the impairment of retinoid signaling [31] and the downregulation of the drug and retinol metabolism genes [32] in HCC. Therefore, our findings consistent with previous reports indicating the downregulation of genes functioning in drug and retinol metabolisms.

The liver cells have crucial roles in amino acid metabolism and protein synthesis [33]. Amino acid metabolisms of BCAAs and tryptophan proposed as targets in cancer growth and anti-tumor immune response. BCAAs are also important for regulation of cellular functions, such as protein translation, cell growth, proliferation, and autophagy [29]. In this study, we observed downregulation of the amino acid metabolism genes (Table 4), which may reflect the functional deterioration of hepatic cells due to carcinogenesis.

Few of the upregulated genes specific to B-CLL annotated to KEGG pathways; "prostate cancer" (gene count = 10) and "melanogenesis" (gene count = 10) (Table 6). At the gene level, many of the genes in these terms, such as CDK2, RAS and WNT, are also the regulators of cell proliferation. Consistent with the phenotype observed in B-CLL patients, we observed downregulation of immune system genes in our analysis (Tables 5 and 8). B cells generally interact with cytokines and T cells. T cells may have an effect on anti-tumor immune responses and anti-tumor T-cell activation decreases in B-CLL cells [34]. In our analysis, we observed downregulation of the genes that were annotated in T-cell activation in both HCC and B-CLL. However, 10 genes involved in T-cell activation were downregulated specifically in B-CLL cells from our microarray data analysis ($p = 0.027$, data not shown). Therefore, it may be inferred that additional mechanisms of T cell activation were affected only in B-CLL cells. We observed downregulation of hematopoietic cell lineage, chemokine signaling pathway, and prion diseases (Table 6), which may be related with the functional defects of immune response in B-CLL. Hematopoietic cell lineages are differentiated forms of hematopoietic stem cells and they are the primary blood cells. Lymphoid lineage of hematopoietic cells forms into T, B and natural killer (NK) cells [35].

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

In this differentiation process, most of the cluster of differentiation (CD) antigens regulate B cell development and especially CD79a (Igα) function in B cell antigen receptor (BCR) signaling, which modulates communication of B cells with extracellular environment [36]. We observed downregulation of several CD molecules, such as CD22, CD72, CD80, CD36 and CD33, in B-CLL cells. CD22 and CD72 negatively regulate BCR signaling [37]. In addition, Prion proteins also functions in transmembrane signaling processes [38]. These antigens and proteins have effects on Hematopoietic cell lineages, chemokine signaling pathway and energy mechanisms [36, 37].

In this study, for the microarray analysis, we selected ANOVA p-value and fold change as selection criteria of the significantly changed probe sets between patient and controls, and did not consider multiple test corrections, such as false discovery rate (FDR). Although some of the probe sets were likely to be false positives when we used p-value (<0.05) as a selection criterion without multi test corrections, this approach increases the number of probe sets in the gene lists. When we consider the FDR p-value (<0.05), ANOVA p-value (<0.05) and FC (linear) <–2 or FC (linear) >2 as selection criteria of the significantly changed probe sets, the number of significantly changed probe sets reduces to 492 from 2339 for HCC. For B-CLL, it reduces to 930 from 1976 probe sets.

Therefore, considering ANOVA p-value (<0.05), without multiple test corrections, allowed us to have larger probe set lists for each cancer type, enabled us to compare the significantly changing genes between two different cancer types, HCC and B-CLL, and to perform KEGG pathway and GO BP analyses.

It has been noted that when a short significant gene list is used, the pathways with intermediate effects may not be detected effectively. Using FDR p-values might be preferred to obtain limited number of genes, when performing confirmations or further sensitive studies [39].

When annotating probe sets to related pathways and BP terms, we reported Benjamini, Benjamini-Hochberg corrected P-value, in addition to p-value (Tables 2–8). However, we considered p-value as only selection criterion for detection of significant KEGG or BP terms, since several significant terms ($p < 0.05$) were lost, when the Benjamini filter was applied.

In conclusion, comparing gene expression profiles of different cancer by bioinformatics is a promising approach to enlighten core mechanisms and also type specific gene expression alterations of cancer cells. In this study, we identified shared gene expression profiles and molecular alterations in HCC and B-CLL cells. Our literature search showed similar behavior of those mechanisms in different cancers. Since HCC and B-CLL originated from different cell lineages, we observed type specific expression changes of large set of genes. Our results revealed that cell function deficiencies due to carcinogenesis were also reflected at the gene expression level. Although, our study contributes to HCC and B-CLL studies, further studies with higher sample sizes are needed for better statistical power to make conclusive remarks on the molecular mechanisms of B-CLL and HCC.

Acknowledgements: Authors are thankful to Hacettepe University, Institute of Health Sciences and Department of Bioinformatics for providing facilities for this study.

Conflict of interest: All authors declare that there is no conflict of interest.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.
2. Janevska D, Chaloska-Ivanova V, Janevski V. Hepatocellular carcinoma: risk factors, diagnosis and treatment. Open Access Maced J Med Sci 2015;3:732–6.
3. Aravalli RN, Steer CJ, Cressman EN. Molecular mechanisms of hepatocellular carcinoma. Hepatology 2008;48:2047–63.
4. Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001;194:1639–47.
5. Gribben JG. How I treat CLL up front. Blood 2010;115:187–97.
6. Fan HB, Zhu YF, Chen AS, Zhou MX, Yan FM, Ma XJ, et al. B-cell clonality in the liver of hepatitis C virus-infected patients. World J Gastroenterol 2009;15:1636–40.
7. Heidecke S, Stippel DL, Hoelscher AH, Wedemeyer I, Dienes HP, Drebber U. Simultaneous occurrence of a hepatocellular carcinoma and a hepatic non-Hodgkin's lymphoma infiltration. World J Hepatol 2010;2:246–50.
8. Kou Y, Zhang S, Chen X, Hu S. Gene expression profile analysis of colorectal cancer to investigate potential mechanisms using bioinformatics. Onco Targets Ther 2015;8:745–52.
9. Pilarsky C, Wenzig M, Specht T, Saeger HD, Grutzmann R. Identification and validation of commonly overexpressed genes in solid tumors by comparison of microarray data. Neoplasia 2004;6:744–50.
10. Reddy RB, Bhat AR, James BL, Govindan SV, Mathew R, Ravindra DR, et al. Meta-analyses of microarray datasets identifies ANO1 and FADD as prognostic markers of head and neck cancer. PLoS One 2016;11:e0147409.
11. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics data sets – update. Nucleic Acids Res 2013;41(Database issue):D991–5.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM

12. Edgar R, Domrachev M, Lash AE. Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002;30:207–10.
13. Kolesnikov N, Hastings E, Keays M, Melnichuk O, Tang YA, Williams E, et al. ArrayExpress update – simplifying data submissions. Nucleic Acids Res 2015;43(Database issue):D1113–6.
14. Roessler S, Lin G, Forgues M, Budhu A, Hoover S, Simpson RM, et al. Integrative genomic and transcriptomic characterization of matched primary and metastatic liver and colorectal carcinoma. Int J Biol Sci 2015;11:88–98.
15. Vargova K, Curik N, Burda P, Basova P, Kulvait V, Pospisil V, et al. MYB transcriptionally regulates the miR-155 host gene in chronic lymphocytic leukemia. Blood 2011;117:3816–25.
16. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 2003;19:185–93.
17. Oliveros JC. Venny. An interactive tool for comparing lists with Venn's diagrams. 2007–2015.
18. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009;4:44–57.
19. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009;37:1–13.
20. de Hoon MJ, Imoto S, Nolan J, Miyano S. Open source clustering software. Bioinformatics 2004;20:1453–4.
21. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 1998;95:14863–8.
22. Saldanha AJ. Java Treeview-extensible visualization of microarray data. Bioinformatics 2004;20:3246–8.
23. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017;45(W1):W98–102.
24. Pirooznia M, Nagarajan V, Deng Y. GeneVenn – a web application for comparing gene lists using Venn diagrams. Bioinformation 2007;1:420–2.
25. Ge X, Yamamoto S, Tsutsumi S, Midorikawa Y, Ihara S, Wang SM, et al. Interpreting expression profiles of cancers by genome-wide survey of breadth of expression in normal tissues. Genomics 2005;86:127–41.
26. Martinez-Ledesma E, Verhaak RG, Trevino V. Identification of a multi-cancer gene expression biomarker for cancer clinical outcomes using a network-based algorithm. Sci Rep 2015;5:11966.
27. Xue J, Yang G, Ding H, Wang P, Wang C. Role of NSC319726 in ovarian cancer based on the bioinformatics analyses. Onco Targets Ther 2015;8:3757–65.
28. Bjornson E, Mukhopadhyay B, Asplund A, Pristovsek N, Cinar R, Romeo S, et al. Stratification of hepatocellular carcinoma patients based on acetate utilization. Cell Rep 2015;13:2014–26.
29. Ananieva E. Targeting amino acid metabolism in cancer growth and anti-tumor immune response. World J Biol Chem 2015;6:281–9.
30. Tonjes M, Barbus S, Park YJ, Wang W, Schlotter M, Lindroth AM, et al. BCAT1 promotes cell proliferation through amino acid catabolism in gliomas carrying wild-type IDH1. Nat Med 2013;19:901–8.
31. Shirakami Y, Sakai H, Shimizu M. Retinoid roles in blocking hepatocellular carcinoma. Hepatobiliary Surg Nutr 2015;4:222–8.
32. Yildiz G, Arslan-Ergul A, Bagislar S, Konu O, Yuzugullu H, Gursoy-Yuzugullu O, et al. Genome-wide transcriptional reorganization associated with senescence-to-immortality switch during human hepatocellular carcinogenesis. PLoS One 2013;8:e64016.
33. Kawaguchi T, Izumi N, Charlton MR, Sata M. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. Hepatology 2011;54:1063–70.
34. Chanan-Khan AA, Chitta K, Ersing N, Paulus A, Masood A, Sher T, et al. Biological effects and clinical significance of lenalidomide-induced tumour flare reaction in patients with chronic lymphocytic leukaemia: in vivo evidence of immune activation and antitumour response. Br J Haematol 2011;155:457–67.
35. Iwasaki H, Akashi K. Hematopoietic developmental pathways: on cellular basis. Oncogene 2007;26:6687–96.
36. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood 2008;112:1570–80.
37. Nitschke L. The role of CD22 and other inhibitory co-receptors in B-cell activation. Curr Opin Immunol 2005;17:290–7.
38. Westergard L, Christensen HM, Harris DA. The cellular prion protein (PrPC): its physiological function and role in disease. Bba-Mol Basis Dis 2007;1772:629–44.
39. Chen JJ, Wang SJ, Tsai CA, Lin CJ. Selection of differentially expressed genes in microarray data analysis. Pharmacogenomics J 2007;7:212–20.

Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/tjb-2018-0039).

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 3/26/19 10:53 AM