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GENE EXPRESSION PROFILING OF B-CLL IN UKRAINIAN PATIENTS IN POST-CHERNOBYL PERIOD

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***Background*: Genetic mechanisms that result in the development and progression of B-cell chronic lymphocytic leukemia (B-CLL) are mainly unknown. We have analyzed gene expression patterns in Ukrainian B-CLL patients with the *aim* of identifying B-CLL involved / associated genes in order to shed light on the biology of this pathological entity. *Material and methods*: The samples of the peripheral blood and bone marrow of 44 Ukrainian B-CLL patients with no characteristics indicative of unfavorable course of the disease such as CD38 were analyzed morphologically and immunocytochemically according to the new WHO classification. Total RNA was isolated, and gene expression levels were determined by microarray method comparing with the sample from 17 healthy donors. *Results*: We investigated interactions using the Ingenuity Pathway Analysis (IPA) software and found 1191 network eligible up-regulated genes and 3398 Functions/Pathways eligible up-regulated genes, 1225 network eligible down-regulated genes and 2657 Functions/Pathways eligible down-regulated genes. *Conclusion*: In B-CLL patients, gene networks around *MYC, HNF1A* and *HNF4A*, *YWHAG*, *NF-κB1* and *SP1* are identified as up-regulated; *CEBPA*, *YWHAG*, *SATB1* and *RB1* — as down-regulated. G protein coupled receptor signaling, arachidonic acid and linoleic acid metabolisms, calcium signaling, metabolism of xenobiotics by cytochrome P450 are found out as significant up-regulated pathways. EIF2 and Cdc42 signaling, regulation of eIF4 and p70S6k signaling, protein ubiquitination pathway and oxidative phosphorylation are the most significant down-regulated pathways obtained in our study. The involvement of *NF-κB* gene network and upregulated levels of G protein coupled receptor signaling pathway, which has an important role in transcription of NF-κB, are important and need further examination.**

***Key Words*: B-CLL, gene expression profiling, microarray analysis, gene networks.**

Ionizing radiation (IR) is one of the most studied carcinogens in the development of multiple myeloma, primary myelofibrosis, polycythemia vera, non-Hodgkin's lymphomas, myelodysplastic syndromes and some forms of acute and chronic leukemia, especially in acute myelogenous leukemia (AML) [1, 2]. Until recently, chronic lymphocytic leukemia (CLL) has not been considered as a radiation-associated leukemia. Nevertheless, current understanding of radiation-induced tumorigenesis and the etiology of lymphatic neoplasia show that IR exposure increases CLL risk [3].

After Chernobyl nuclear accident, people living in the contaminated areas of Ukraine are still exposed to low doses of IR. Analysis of the patients with various forms of the malignancies of hematopoietic and lymphoid tissues has not revealed the differences in B-CLL percentage among Chernobyl clean-up workers and Ukrainian population in whole. B-CLL was shown to be a predominant form of hematopoietic malignancies in clean-up workers as well as in general population [4]. Genetic mechanisms that result in the development and progression of CLL are mainly unknown [5]. Gene expression profiling by microarray is useful to understand B-CLL origin and development [6]. The analysis of the molecular genetic features should be advantageous in elucidating the putative association of IR and B-CLL.

Earlier, we have studied gene expressions of several apoptosis related genes in different types of tumors of hematopoietic and lymphoid tissues in 189 patients including those with B-CLL living in areas of Ukraine contaminated with radionuclides in post-Chernobyl period [7]. In the present study, we have analyzed gene expression patterns in samples from 44 B-CLL Ukrainian patients in post-Chernobyl period with the aim of identifying the genes related to or involved in this pathology in order to shed light on the biology of B-CLL.

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Abbreviations used: AML – acute myelogenous leukemia; B-CLL – B-cell chronic lymphocytic leukemia; IPA – Ingenuity Pathway Analysis; IR – ionizing radiation.

MATERIAL AND METHODS

The samples of the peripheral blood of B-CLL patients were obtained from R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine. All the patients were referred to Reference Laboratory of Immunocytochemistry and Oncohematology Department of the Institute for verifying the diagnosis. Bone marrow and peripheral blood smears stained by May-Grunwald-Giemsa were studied morphologically. Immunocytochemical techniques (APAAP, LSAB-AP) and a broad panel of monoclonal antibodies against lineage specific, differentiation and activation antigens of leukocytes were employed for immunophenotyping pathological cells in blood and bone marrow. The main forms and cytological variants of hematological malignancies were diagnosed according to new WHO classification [8]. All the samples were immunophenotyped, and only 44 samples from CD38-negative B-CLL patients out of 127 diagnosed patients with B-CLL/B-cell lymphoma from small lymphocytes [7] were included in the study. Control group comprised peripheral blood samples from 17 healthy donors. The ethic committees of both collaborating research institutions approved the design of the study.

***Total RNA isolation*.** Total RNA was isolated from mononuclear cells for each patient using QIAamp RNA Blood Mini Kit (QIAGEN, Valencia, CA, USA) and treated

with DNase I according to the manufacturer's instructions. The quality of the RNA was assessed by loading 300 ng of total RNA onto an RNA Labchip (Agilent Technologies, Waldbronn, Germany), followed by analysis (A2100 Bioanalyzer; Agilent Technologies). An RNA integrity value (RIN) of 7.0 was considered acceptable.

RNAs from 44 B-CLL patients and 17 healthy donors were pooled seperately. Pooling process was performed in the way that 100 ng RNA sample was used from each B-CLL patient/healthy donor. Each RNA pool was prepared as three replicates.

Microarray analysis. Microarray analysis was performed using the Whole Human Genome Oligo Microarray (Agilent Technologies), encompassing more than 44,000 human DNA probes. The full list of cDNAs is available online (www.agilent.com). Protocols for sample preparation and hybridization of the mononuclear cells were adaptations of those in the Agilent Technical Manual. In short, first strand cDNA was transcribed from 300 ng of total RNA using T7-Oligo(dT) Promoter Primer. Samples were transcribed *in vitro* and Cy-3-labelled by using a Quick-AMP labeling kit (Agilent Technologies). Following a further clean-up round (Qiagen), cRNA was fragmented into pieces ranging from 35 to 200 bases in size. Fragmented cRNA samples (1.65 mg) were hybridized onto chips by means of 17 h of incubation at 65°C with constant rotation, followed by a two-step microarray wash of 1 min in two washing buffers (Agilent Technologies). Hybridized microarrays were scanned in a Agilent Technologies Scanner (model G2505B) and numerical results were extracted with Feature Extraction version 9.5.1.1 using 014850_D_F_20060807 grid, GE1-v5_95_Feb07 protocol and GE1_QCM_Feb07 QC metric set.

The microarray data were analyzed using GeneSpring software version 9.0 (Agilent Technologies, Santa Clara, CA). The fold changes were analyzed by filtering the dataset using P-value < 0.01 and a signal-to-noise ratio >2 for use in T-test statistical analysis. Additional filtering (minimum 2-fold change) was applied to extract the most these genes, which were analyzed using Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, Redwood City, CA). Those genes with known gene symbols (HUGO) and their corresponding expression values were uploaded into the software. Each gene symbol was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base. Networks of these genes were algorithmically generated based on their connectivity and assigned a score. The score is a numerical value used to rank Networks according to how relevant they are to the genes in the input dataset but may not be an indication of the quality or significance of the network. The score takes into account the number of focus genes in the network and the size of the network to approximate how relevant this network is to the original list of focus genes. The network identified is then presented as a graph indicating the molecular relationships between genes/gene products. Genes are represented as nodes, and the biological relationship between two nodes is represented as an edge (line). The intensity of the node color indicated the degree of up- or down-regulation. The node shapes are disclosed in corresponding figure legends. Canonical pathway analysis identified the pathways from the IPA library of canonical pathways, which were most significant to the input data set. The significance of the association between the data set and the canonical pathway was determined based on two parameters: (1) A ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway and (2) a P value calculated using Fischer's exact test determining the probability that the association between the genes in the data set and the canonical pathway is due to chance alone.

Quantitative real-time PCR (Q-RT-PCR). cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Fermentas Inc., Maryland, USA). Q-RT-PCR was performed as we described previously for determination of *MYC*, *BAX*, *BCL-2* and *FAS1* gene expressions [9, 10]. Standard curves were obtained using serial dilutions of the beta-globulin gene (DNA Control Kit, Roche). Gene-specific primers (Table 1) were obtained from Integrated DNA Technologies (Iowa, USA). Obtained gene expression values were normalized using a housekeeping gene of beta2 microglobulin. Gene expression ratios were compared in patient and control groups using REST (Relative Expression Software Tool).

Table 1. List of the primers used for the quantitative RT-PCR

Genes	Primer sequences
Beta2 microglobulin	(F) 5' TGA CTT TGT CAC AGC CCA AGA TA 3'
	(R) 5' AAT CCA AAT GCG GCA TCT TC 3'
BAX	(F) 5' TGC TTC AGG GTT TCA TCC AG 3'
	(R) 5' GGC GGC AAT CAT CCT CTG 3'
MYC	(F) 5' GGC AAA AGG TCA GAG TCT GG 3'
	(R) 5' GTG CAT TTT CGG TTG TTG C 3'
FAS1	(F) 5' CAA GGG ATT GGA ATT GAG CA 3'
	(R) 5' GAC AAA GCC ACC CCA AGT TA 3'
BCL-2	(F) 5' AGG AAG TGA ACA TTT CGG TGA C 3'
	(R) 5' GCT CAG TTC CAG GAC CAG GC 3'

RESULTS

Differentially expressed genes are shown in two separate tables. The 100 most up-regulated genes are shown in Table 2. The 100 most down-regulated genes are shown in Table 3. Both sets of results were obtained based on minimum 2-fold change using GeneSpring software version 9.0 (Agilent Technologies, Santa Clara, CA). In Table 4 the gene expression results of four genes (*MYC*, *BAX*, *BCL-2* and *FAS1*) obtained by real-time PCR and microarray methods are compared. Real-time PCR results of *MYC*, *BCL-2* and *BAX* are in a good agreement with microarray expression rates.

Table 4. Summarized real-time PCR confirmation results of the four genes

Genes	Ratios obtained by RT-PCR	Ratios obtained by arrays
BAX	5.0265 (Up-regulated)	2.592 (Up-regulated)
BCL-2	16.696 (Up-regulated)	1.747 (Up-regulated)
MYC	4.15 (Up-regulated)	2.794 (Up-regulated)
FAS1	4.536 (Up-regulated)	2.460 (Down-regulated)

We investigated interactions using IPA software and found 1191 network eligible up-regulated genes and 3398 Functions/Pathways eligible up-regulated genes. Fig. 1 shows the most significant four gene networks of over-expressed genes in B-CLL samples. Top functions of these genes are related to hematopoiesis, lipid metabolism, small molecule biochemistry, cancer, infec-

Table 2. The 100 most up-regulated genes in B-CLL

Fold Change	Gene	Fold Change	Gene
9.971721	CB162722	7.9061837	TTC23
9.893506	THC2579650	7.886104	SFRP1
9.856071	IRX5	7.8818917	FLJ32679
9.828577	SAPS1	7.8160353	MMP14
9.718567	THC2671344	7.798868	MEGF10
9.659609	LRRC2	7.7877035	WDR21C
9.598212	PIGR	7.775479	BU587941
9.52783	BX119852	7.7426653	BCR
9.385712	FMOD	7.7220807	THC2676656
9.34002	CGB1	7.706189	AI089002
9.180631	VPS18	7.6771984	WNT3
9.170944	RAPH1	7.648338	UCP3
9.11223	RNF150	7.647829	NFE2L1
9.11183	RAP1GAP	7.6217384	C1orf168
9.109504	RPA4	7.6014295	TMPRSS3
9.073619	THC2672701	7.6004906	WNT2B
9.049471	CD86	7.5972705	TUSC5
9.029652	RBM22	7.5422063	TEX12
8.81397	AA704712	7.522491	MGC88374
8.759307	AA479896	7.4850636	ST6GAL1
8.743843	AKAP12	7.4668427	LOC645478
8.691222	CCDC66	7.4543867	KIAA0672
8.670482	ABCA4	7.4285965	NAV2
8.608516	CV575560	7.419999	THC2537502
8.573189	GRAMD1C	7.419809	KIAA1946
8.567822	EFTUD1	7.3947935	BX647159
8.518443	LOC389043	7.3545713	BG190682
8.484631	S71486	7.3339643	RUNDC2B
8.467656	BTC	7.3178434	GBP6
8.455834	SMARCA4	7.2903414	ZNF713
8.4216795	MGC39584	7.2862663	ASB16
8.39463	BF368414	7.2639813	THC2530551
8.346779	C1orf173	7.2611523	PPM1F
8.317559	NDP	7.2371364	MYOC
8.281372	BI826226	7.228985	LOC643401
8.207127	RPTN	7.2250643	KALRN
8.186712	PRRX1	7.215619	MYCNOS
8.142795	BQ286187	7.1989717	CRISPLD2
8.100048	L5	7.1989717	CRISPLD2
8.054283	ATXN3L	7.192935	ADIPOQ
8.05317	AK098548	7.192935	ADIPOQ
8.044337	TEF	7.1847763	SLC44A5
8.034349	WDR33	7.1847763	SLC44A5
8.031527	CASKIN2	7.1711025	ZCCHC13
8.008858	FLJ25770	7.135996	SLC27A1
7.9823356	THC2686753	7.1255236	ZNF2
7.9713397	KLHL23	7.122238	MSTP9
7.9610386	POLR2J2	7.0874977	PSPH
7.9588156	STARD13	7.048849	PYY2
7.950879	MLL	7.032443	AD7C-NTP

Table 3. The 100 most down-regulated genes in B-CLL

Fold Change	Gene	Fold Change	Gene
-9.467819	THC2588392	-6.4505854	BCL2A1
-8.866756	HBG1	-6.4489126	TTRAP
-8.638458	SELENBP1	-6.3537326	TNFAIP2
-8.50988	HBA2	-6.341367	IL1R2
-8.47693	HBG1	-6.3040967	FYB
-7.8862677	SAT1	-6.26194	S100A12
-7.8419037	FCGR3A	-6.2470803	TLR2
-7.80179	RGS2	-6.2420635	SNCA
-7.7845144	SLC25A39	-6.2413063	PBEF1
-7.7299724	ALAS2	-6.2392893	THC2586959
-7.686287	KRT1	-6.231715	CAMP
-7.6433597	SRGN	-6.2299414	S100A8
-7.6020937	PROK2	-6.2271876	KRT23
-7.5707283	S100P	-6.193751	DYNLT1
-7.5505257	TNFRSF10C	-6.171741	SLC31A2
-7.475219	MXD1	-6.153518	RGS18
-7.389961	HBD	-6.139215	SIPA1L1
-7.376893	CLEC4E	-6.125804	CCR1
-7.297138	CMTM6	-6.0938168	ADD3
-7.2936077	FTL	-6.021562	NFE2
-7.292986	PAIP2	-6.0161657	QPCT
-7.2235703	ALAS2	-5.994034	ITM2B
-7.182671	HBD	-5.9857407	YPEL5
-7.1385164	LGALS3	-5.9691944	IFNGR1
-7.1038146	IFIT2	-5.955679	IL8RB
-7.0883236	ANXA1	-5.950643	C20orf24
-7.055394	AQP9	-5.9466496	GLUL
-7.054615	LOC552891	-5.9364004	NINJ1
-6.935093	C6orf32	-5.9354315	C5orf32
-6.9139557	PDZK1IP1	-5.9249115	VPS4B
-6.892113	FBXL5	-5.9206657	FLJ10357
-6.8429413	CMTM2	-5.9169197	HSD17B11
-6.823658	HBQ1	-5.904073	UBB
-6.8207946	BNIP3L	-5.895618	FTL
-6.7675858	CLC	-5.894103	SAT1
-6.7639685	AP1S2	-5.8842864	CKLF
-6.7029543	ALOX5AP	-5.8623157	MYL4
-6.678584	ACTG1	-5.8620443	FBXO7
-6.6524496	GIMAP7	-5.8529325	LCP1
-6.643402	GCA	-5.8372726	SNN
-6.632475	CSTA	-5.8210387	BNIP3L
-6.6212616	PBEF1	-5.8020077	MTPN
-6.5431356	LIMK2	-5.7948103	COPS5
-6.537367	SOD2	-5.7918744	NGFRAP1
-6.535038	TP53INP1	-5.782423	MFSD1
-6.5181375	IFIT1	-5.7802	MPP1
-6.475131	BID	-5.7671204	HIPK1
-6.470724	HIST1H2AC	-5.7636905	PBEF1
-6.470461	DUSP1	-5.746536	PAG1
-6.461632	MNDA	-5.7328815	APOBEC3A

tious diseases, cell cycle, cardiovascular system development and function, gene expression, embryonic development, tissue morphology, inflammatory response. Up-regulated gene networks are identified around *MYC*, *HNF1A* and *HNF4A*, *YWHAG*, *NF-κB1* and *SP1*.

We also found 1225 network eligible down-regulated genes, and 2657 Functions/Pathways eligible down-regulated genes. Fig. 2 shows four gene networks of down-regulated genes in B-CLL. The main functions of the genes are related to cellular functions and maintenance, protein synthesis, dermatological diseases and conditions, cell death, gene expression, inflammatory disease, cellular growth and proliferation, post-translational modification, cancer, infectious diseases, cell morphology, and development. Down-regulated gene networks are identified around *CEBPA*, *YWHAG*, *SATB1* and *RB1*.

DISCUSSION

B-CLL is a heterogeneous disease and a predominant form of hematopoietic malignancies. Despite new molecular methods identifying important prognostic and diagnostic genetic markers, genetic mechanisms involved in B-CLL origin are mainly unknown. A number of novel prognostic markers such as Bcl-2, MAP-kinase, NF-κB, ZAP-70 were identified applying gene expression profiling before [11, 12].

We have analyzed gene expression patterns in samples from 44 B-CLL Ukrainian patients in post-Chernobyl period to identify genes associated with this form of lymphoproliferative malignancy. Our study has demonstrated new genetic networks and biological pathways in both up- and down-regulated gene expression levels.

Analysis using IPA software revealed 1191 network eligible up-regulated genes and 3398 Functions/Pathways eligible up-regulated genes. The individual genes are found in multiple categories of functions related to hematopoiesis, lipid metabolism, small molecule biochemistry, cancer, infectious diseases, cell cycle, development and function of cardiovascular system, gene expression, embryonic development, tissue morphology, inflammatory response.

One important gene network is identified around the up-regulated *MYC* and *SP1* genes (Fig. 1, *a*). *MYC*, a strong proto-oncogene, plays very important roles in cell proliferation (by upregulating cyclins, downregulating p21), controlling cell growth (by upregulating ribosomal RNA and proteins), apoptosis (by downregulating *BCL-*

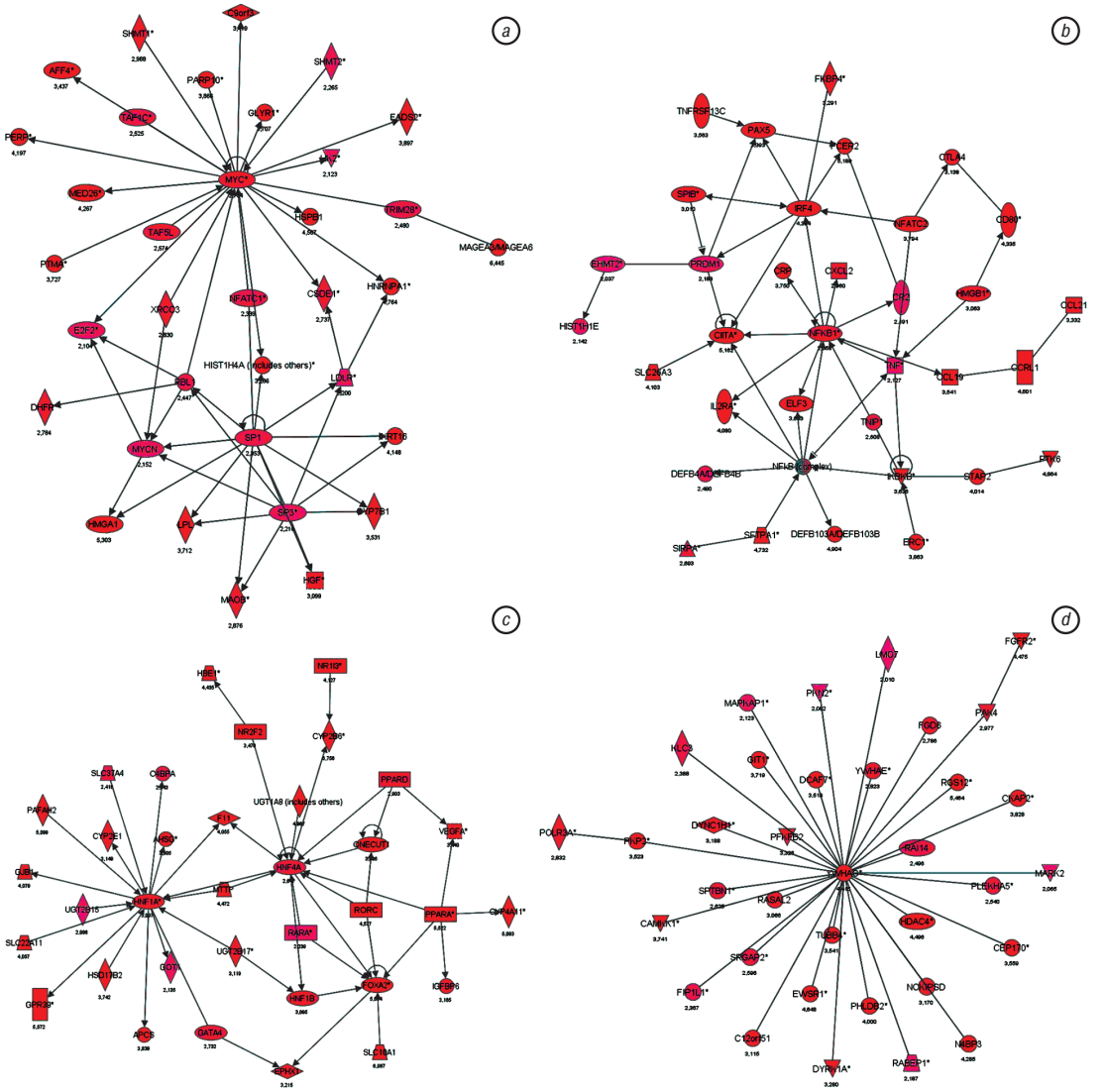

Fig. 1. Significant up-regulated gene networks identified around *MYC* and *SP1* genes (*a*), *NF-κB1* gene (*b*), *HNF1A* and *HNF4A* genes (*c*), *YWHAG* gene (*d*) in B-CLL samples. The node shapes denote enzymes (◇), phosphatases (△), kinases (▽), peptidases (◇), G-protein coupled receptor (▯), transmembrane receptor (⬯), cytokines (□), growth factor (⬚), ion channel (⬚), transporter (⏢), translation factor (⬭), nuclear receptor (▭), transcription factor (⬭) and other (○).The intensity of the node color-(*red*) indicated the degree of up-regulation

2), differentiation and stem cell self-renewal. Mutations, overexpression, rearrangement and translocation of *MYC* have been associated with a variety of hematopoietic tumors, leukemias and lymphomas, including Burkitt lymphoma [13]. High expression level of *MYC* has been reported in more aggressive and apoptosis resistant forms of B-CLL and might be used as molecular marker specific of resistant B-CLL subsets [14, 15]. SP1, a zinc finger transcription factor, is involved in cell differentiation, cell growth, apoptosis, immune response, response to DNA damage, and chromatin remodeling. *SP1* and *MYC* are involved cooperatively in telomerase activation, which is a critical step in cellular immortalization and carcinogenesis. Kyo *et al.* have suggested that the level of SP1 expression might be a critical determinant of telomerase activity both in cancer and normal cells [16].

Another network is identified around *NF-κB1* gene (Fig. 1, *b*). NF-κB regulates several genes that mediate tumorigenesis and metastasis and also plays an important role in pathogenesis of B-cell neoplasms. Carcinogens, tumor promoters, inflammatory cytokines, and chemotherapeutic agents activate *NF-κB* and this activation can suppress apoptosis, thus promoting chemoresistance and tumorigenesis. Bharti *et al.* suggested that NF-κB might be an ideal target for chemoprevention and chemosensitization [17, 18]. In addition, we have found *NF-κB* gene centered around two up- and down-regulated networks in our previous study on prostate cancer [19].

Canonical pathway analysis revealed that G-protein coupled receptor (GPCR) signaling is an important pathway modulated by the up-regulated genes in B-CLL. It is known that GPCRs regulate proliferation, differentiation, chemotaxis and also they play an important role in inflammatory diseases and cancer [20]. GPCRs are involved in control of transcription factors such as STAT3, NF-κB and CREB by G protein subfamilies [21]. Enhanced viability of CLL cells by the STAT3 phosphorylation and interaction between hepatocyte growth factor and its receptor (c-MET), which

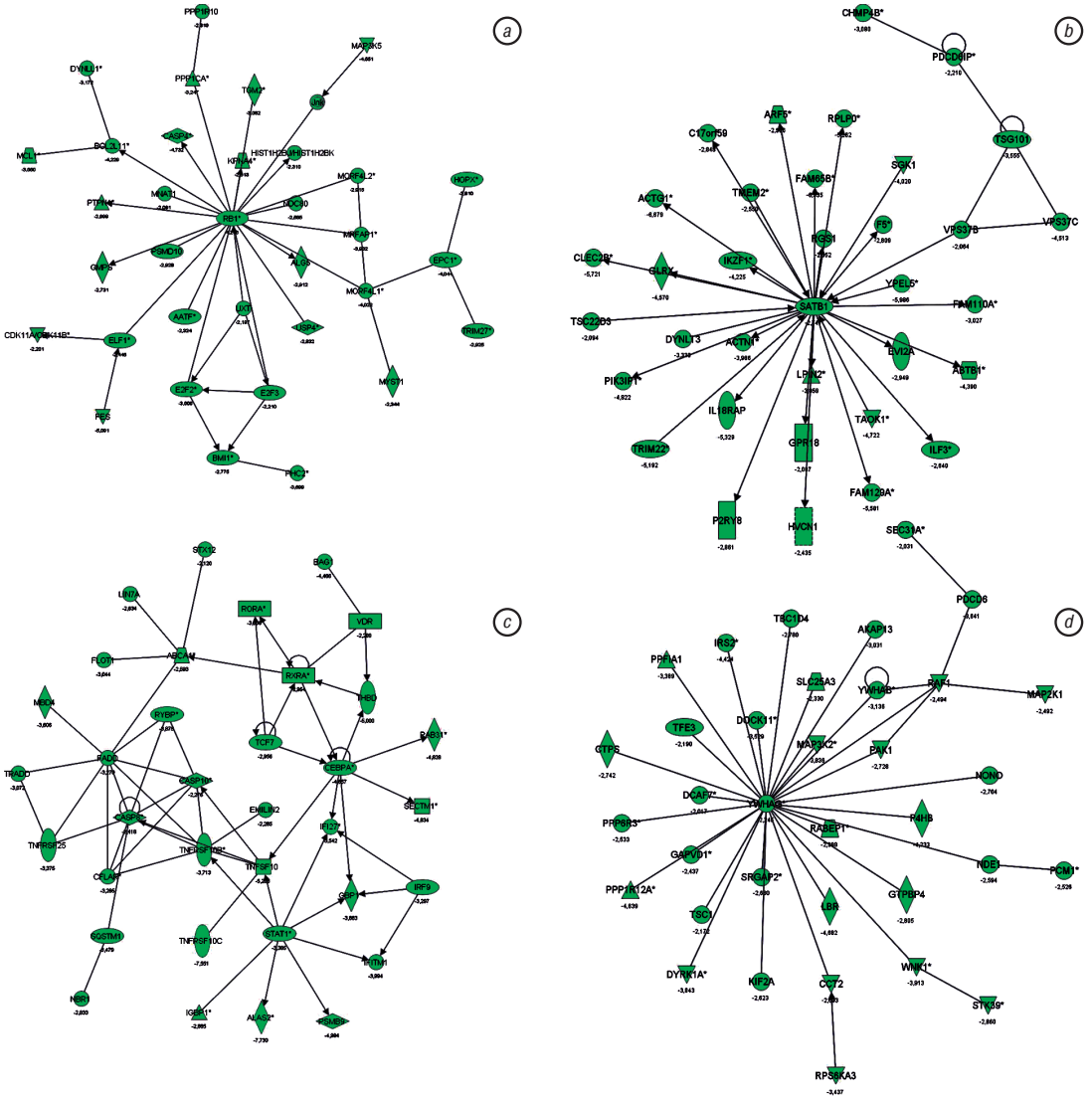

Fig. 2. A significant down-regulated gene network identified around *RB1* gene (*a*), *SATB1* gene (*b*) in B-CLL samples, *CEBPA* gene (*c*), *YWHAG* gene (*d*) in B-CLL samples. The keys to the node shapes are the same as in Fig. 1. The intensity of the node color-(*green*) indicates the degree of down-regulation

was found up-regulated in our study, was reported before [22]. CREB (cAMP response element binding protein) had been found overexpressed in bone marrow samples from patients with acute lymphoid and myeloid leukemia and associated with a poor outcome in AML patients according to previous studies [23].

A network is also identified around *HNF1A* and *HNF4A* (Fig. 1, *c*). HNF1A is a transcription factor required for the expression of several liver-specific genes and the expression of this gene is controlled by HNF4A, which may play role in development of the liver, kidney and intestines.

Another significant signaling pathway is calcium signaling involved in many processes such as cell survival/apoptosis, cell cycle progression, differentiation, cross-talk between intracellular compartments (ER, mitochondria), general metabolism and telomerase activity. The calcineurin/NFAT signaling pathway is important in lymphoma/leukemogenesis [24]. Deregulation of this signaling and/or abnormal expression of its components has been reported in solid tumors of epithelial origin, lymphoma and lymphoid leukemia. Mouse models of human T-ALL/lymphoma showed the pro-oncogenic effect of active calcineurin/NFAT signaling *in vivo* [25]. NFAT transcription factors form four calcium signaling responsive members: NFATc1, NFATc2, NFATc3 and NFATc4. Among these members NFATc1 and NFATc2, which are found up-regulated in our study, were reported to be involved in the development, differentiation and functioning of multiple T-and B-cell subsets in previous studies. NFATc1 was found to be expressed in a majority of aggressive B-cell lymphomas. On the other hand, NFATc2 activation was shown to be responsible in B-CLL, in cooperation with STAT6, for the high expression of CD23 [24].

Metabolism of xenobiotics by cytochrome P450 pathway has been shown as highly significant in our study. The enhanced expression of several P450s like CYP1A, CYP2C and CYP3A, that are up-regulated in our study, was reported in tumor cells elsewhere [26].

Arachidonic acid and linoleic acid metabolisms are the other significant pathways modulated by the up-regulated genes in our study.

Analysis using IPA software revealed 1225 network eligible down-regulated genes, and 2657 Functions/Pathways eligible down-regulated genes. These individual

genes are related to cell functions and maintenance, protein synthesis, dermatological diseases and conditions, cell death, gene expression, inflammatory disease, cell growth and proliferation, post-translational modification, cancer, infectious diseases, cell morphology, and development.

One important down-regulated network is identified around *RB1* gene (Fig. 2, *a*). The role of *RB1* in B-CLL has been reported based on cytogenetic data [27]. *RB1* deletions involved in 13q14 abnormalities have been reported in B-CLL before [28].

Another down-regulated network is identified around *SATB1* gene (Fig. 2, *b*). *SATB1* is a new type of gene regulator expressing in various human cancers and thought to be related to the malignant potential. Overexpression of this gene has been reported as a predictor of poor prognosis in lung and gastric cancers [29, 30].

An important network is identified around *CEBPA* gene (Fig. 2, *c*). CEBPA is a critical transcriptional factor and regulates the balance between cell proliferation and differentiation during early hematopoietic development and myeloid differentiation [31]. CEBPA has a tumor-suppressor function in leukemogenesis and both loss of function and gain of function have leukemogenic potential. It was reported that overexpression of CEBPA could contribute to B-ALL and loss of function could contribute to AML [32]. On the other hand, down-regulated CEBPA was found in acute promyelocytic leukemia stem cells in animal models [33].

Canonical pathway analysis revealed that oxidative phosphorylation is an important pathway modulated by the down-regulated genes in B-CLL. In fact, previous studies suggested that the oxidative phosphorylation (OXPHOS) system is severely compromised in various cancers [34].

EIF2 signaling is another significant pathway. Suppression of head and neck, colorectal carcinoma and multiple myeloma tumor growth and/or survival by phosphorylation of eIF2α was reported before [35].

IPA reveals regulation of eIF4 and p70s6K signaling pathway. eIF4E down-regulated in our study plays an important role in tumor initiation and progression when its overexpression cooperates with oncogenes to accelerate transformation in cell lines and animal models [36]. p70s6K is a serine/threonine kinase and its target substrate is S6 ribosomal protein [37]. Inhibition of p70s6K was related to cell cycle arrest at G0/G1 phase in human cancer cells before [38].

Protein ubiquitination is another pathway found significant in our study. Ubiquitination of key signaling molecules by E3 ubiquitin ligases forms an important regulatory mechanism for NF-κB signaling. Deubiquitinases (DUBs) counteract E3 ligases and play a substantial role in down-regulation of NF-κB signaling and homeostasis [39].

Cdc42 signaling is a highly significant pathway. Cdc42 promotes or inhibits tumor progression depending on the cellular context and contributes to cancer development through its different roles in intracellular trafficking, cell cycle regulation and survival, polarity, migration and transcriptional control [40]. Cdc42 is also important in the development and progression of lymphoma. Genetic knockdown or pharmacological inhibition of Cdc42 resulting in a cell cycle arrest and apoptosis of anaplastic large cell lymphoma cells has been reported [41].

An important network is identified around both down-regulated and up-regulated *YWHAG* gene in our study (Fig. 1 and Fig. 2). This gene encoding for 14–3-3 protein gamma was found highly expressed in skeletal and heart muscles. It has been suggested that this protein has an important role in muscle tissue [42, 43]. 14–3-3 proteins play critical regulatory roles in signaling pathways in cell division and apoptosis [44]. Further investigations are required to establish the function of *YWHAG* gene in B-CLL.

Real-time PCR confirmation results of four genes (*MYC*, *FAS1*, *BAX* and *BCL-2*) show that only *MYC*, *BAX* and *BCL-2* expressions are in agreement with microarray results. Up-regulation of *MYC* is compatible with our expectations.

Previous studies indicate that high ratio of Bcl-2 to Bax proteins confers a poor prognosis with decreased rates of complete remission and overall survival [45]. In our study, *BCL-2* upregulation level is superior to that of *BAX* in real-time PCR results but not in microarrays being analyzed.

FAS1 expression was found up-regulated in real-time PCR but down-regulated in microarrays in our study. It has been reported that Fas expression is not very high in B-CLL [46] that coincides with our findings of relatively small up-regulation by real-time PCR. It should be noted that Fas was mentioned as apoptosis regulator [47] in CLL cells exposed to IR.

NF-κB gene network was conspicuous in terms of being determined also in our previous studies of gene expression in prostate cancer. In addition, upregulated levels of G protein coupled receptor signaling pathway, which has an important role in transcription of NF-κB, need advanced examinations. In this sense, *NF-κB* gene which is important in both cell cycle regulation and cancer progression deserves further study.

Our study has presented the gene expression profiling in B-CLL patients of Ukrainian population as whole. We believe that the contribution of IR as the putative factor in the origin of B-CLL should be further evaluated using such molecular genetic approach.

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