

ORIGINAL ARTICLE

# Circulating microRNA-192 as a diagnostic biomarker in human chronic lymphocytic leukemia

S Fathollahzadeh<sup>1</sup>, H Mirzaei<sup>2</sup>, MA Honardoost<sup>3</sup>, A Sahebkar<sup>4</sup> and M Salehi<sup>5</sup>

Chronic lymphocytic leukemia (CLL) is known as the most common lymphoid malignancy in the Western world. MicroRNAs (miRNAs) are a class of small noncoding RNAs with pivotal roles in cellular and molecular processes related to different malignancies including CLL. Recently, some studies have shown that miR-192 plays a key role in CLL pathogenesis through increasing CDKN1A/p21 levels, suppression of Bcl-2 and enhancement of wild-type P53 and cell cycle arrest. Forty samples, including 20 patients with CLL, diagnosed in Omid hospital (Isfahan, Iran) and 20 healthy controls were sampled during a period of 4 months. Using real-time PCR method, expression of miR-192 was analyzed in peripheral blood mononuclear cells (PBMCs) of CLL patients in comparison with healthy subjects. *In silico* molecular signaling pathway enrichment analysis was also performed on validated and predicted targets (targetome) of miR-192 in DAVID database to explore possible role of miR-192 in some pathways. The expression of miR-192 was found to be significantly reduced (~2.5-folds) in CLL patients compared with healthy subjects ( $P=0.002$ ). *In silico* molecular signaling pathway enrichment analysis detected cell indicated signaling pathway as one of the most statistically relevant pathway with miR-192 targetome. Our findings showed that miR-192 could be a biomarker for early diagnosis of CLL.

Cancer Gene Therapy (2016) 23, 327–332; doi:10.1038/cgt.2016.34; published online 16 September 2016

## INTRODUCTION

Chronic lymphoid leukemia (CLL) is known as the most frequent type of leukemia in adults. CLL is characterized by mature-appearing monoclonal B cells with CD5, CD19, CD23 markers and reduction of membrane-bound IgM and IgD.<sup>1</sup> CLL is the most common form of leukemia, accounting for ~30% of all cases. Similar to other cancers, the lack of early symptoms results in late detection of the disease and adverse sequelae.<sup>2</sup> B-lymphocytes mature in bone marrow through rearrangement of immunoglobulin variable (V) gene segments which results in the formation of the code for an immunoglobulin molecule, used as the *B-cell* antigen receptor. This process includes rearrangement of heavy fragments of the *VDJ* gene that encodes the binding site of the receptor.<sup>2,3</sup> According to previous investigations, prognostic prediction in CLL is based on V-gene mutations, CD38, ZAP-70, deletion of chromosome. Expansion and somatic hypermutation of post-germinal center (memory) B cells are the most reported causes of IgVh mutation, and is the most crucial factor in this disease.<sup>3–5</sup> In addition, various types of chromosomal deletions, such as 17q13, 6q21, 11q23, 13q14 and 17p13, have been observed in CLL patients.<sup>5–7</sup> Dysfunction of *TP53* due to regional deletion of chromosome 17 (17p13) has been reported to be associated with resistance to therapy with purine analogs and alkylating agents in CLL. Furthermore, *ATM* gene deletion on chromosome 11 (11q22) increases MDM2 activity which in turn decreases p53 pathway activity in CLL.<sup>8</sup>

MiRNAs are a class of small noncoding RNAs about 21–25 nucleotides long that regulate gene expression post-transcriptionally by binding to the 3'-untranslated region (UTR) of their mRNA

targets, resulting in degradation or transcriptional repression of the targeted mRNA.<sup>9–11</sup> MiRNAs control numerous cellular/molecular processes such as apoptosis, cell proliferation and differentiation.<sup>12,13</sup> Deregulated expression of miRNAs contributes to the initiation or development of numerous diseases such as cancer.<sup>10,12</sup> The role of miRNAs has been previously reported in cell cycle control, in a synergism with some transcription factors, namely c-myc, E2F or P53.<sup>14</sup> miR-192 has been suggested to be a positive regulator of p53. This miRNA has a significant impact on cell differentiation, proliferation and apoptosis.<sup>2,15,16</sup> Several studies have reported deregulated expression of miR-192 in biological samples from cancer patients.<sup>17,18</sup> However, only two studies have reported involvement of miR-192 in CLL: Georges *et al.* compared miR-192 expression in CLL versus CD5<sup>+</sup> cells and Di Liso *et al.* assessed differentially expressed miRNAs in each type of lymphoma.<sup>19,20</sup>

In the current study, the expression of miR-192 was analyzed in peripheral blood mononuclear cells (PBMCs) of CLL patients in comparison with healthy subjects in order to determine whether deregulated expression of this miRNA can be used as a diagnostic biomarker. Furthermore, pathway enrichment analysis was performed to unravel the possible role of miR-192 in the pathogenesis of CLL, and relevant signaling pathways which are affected by this miRNA in CLL.

## MATERIALS AND METHODS

### Diagnostic tests

*Patients and samples.* Blood samples were taken from patients with early-stage disease and random samples from healthy men and women.

<sup>1</sup>Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Yazd, Iran; <sup>2</sup>Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; <sup>3</sup>Division of Cell and Molecular Biology, Department of Biology, Faculty of Science, University of Isfahan, Isfahan, Iran; <sup>4</sup>Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran and <sup>5</sup>Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. Correspondence: Dr M Salehi, Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan 91779-48564, Iran.

E-mail: m\_salehi@med.mui.ac.ir

Received 29 April 2016; revised 25 May 2016; accepted 2 June 2016; published online 16 September 2016

Forty samples, from 20 patients with CLL, diagnosed in the Omid Hospital (Isfahan, Iran), and 20 healthy controls were sampled during a period of 4 months, according to the Internal Review and the Ethics Boards of the Isfahan University of Medical Sciences. (Table 1). Exclusion criteria were: (i) CLL diagnosis more than 12 months before registration; (ii) clinical Binet stage B or stage C; (iii) need of therapy according to NCI guidelines; and (iv) age > 70 years.<sup>21</sup> After informed consent was obtained from the participants, 4 ml blood was collected in EDTA-containing tubes. Immediately after collection, the blood samples were transferred to a laboratory on ice. The Ethics Committee of the Omid Hospital approved the protocol of this study.

**Complete blood count**

This test was performed using CA&XN-Series TM Automated Hematology Analyses. The Sysmex XN-Series uses fluorescence flow cytometry and three-dimensional highly sensitive flagging. This device determines nucleated red blood cells, white blood cell abnormalities, immature granulocytes, and, platelet immature cells and detects atypical lymphocytes. Stages of disease progression were seen in the complete blood count test in three CLL patients with this device as shown in Table 2 and Figure 1.

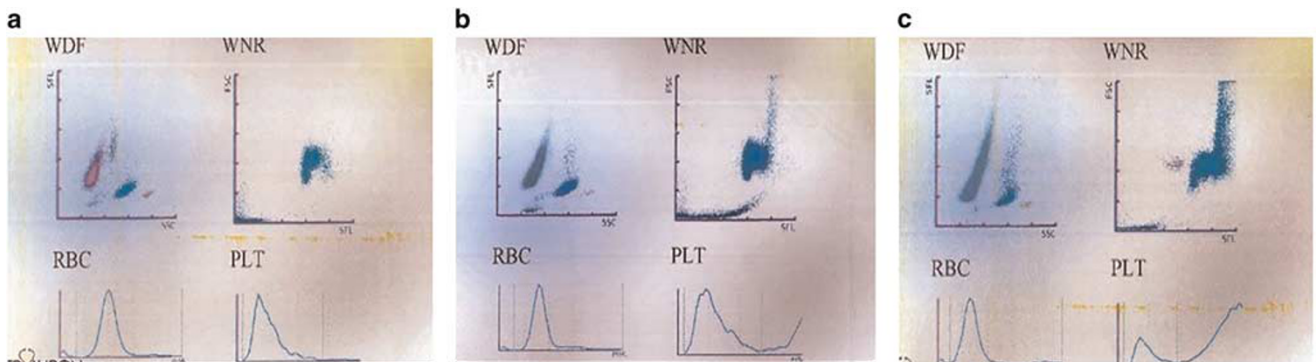
*Peripheral blood mononuclear cells (PBMCs) isolation from blood.* PBMCs were separated by density gradient lymphoprep (Bio Sera, Kansas City, USA) according to the manufacturer's protocol. Because mononuclear cells, monocytes and lymphocytes have lower density in comparison with erythrocytes and leukocytes (granulocytes), after centrifugation, they remain in an intermediate phase, while other cells are deposited. Four ml of blood was diluted at a ratio of 1:1 with physiological saline and gradually added to the 4 ml lymphoprep solution gradient in a falcon tube. Prepared falcons were centrifuged at 800 g for 30 min at room temperature, and then PBMCs were transferred from the middle phase into a 2 ml RNAase-free micro tube. After washing and cell counting, PBMCs were sedimented using centrifugation for 10 min at 250 g and were then frozen at -70 °C until the RNA extraction stage.

*RNA extraction.* Total RNA from PBMCs was extracted using miRNA Hybrid-R (Geneall, Seoul, Korea) based on the manufacturer's instructions. Quality of total RNA was determined at a 260/280 nm wavelength ratio measured by a NanoDrop spectrometer (Thermo Scientific, Waltham, MA, USA).

*cDNA synthesis and real-time PCR.* cDNA synthesis of miR-192 was performed using a commercial kit (Pars Genome, Tehran, Iran) and

**Table 1.** Symptoms, clinical and biological features of patients included in this study

Clinical signs	Complete blood count test results	Disease duration (year)	Age	Sex	Patient code
Drowsiness, swelling of face, diarrhea	Low platelet	3	81	F	a
Dizziness and headache	High white blood cell	2	51	F	b
High platelet	High white blood cell and lymphocyte	1	64	M	c
Excessive sweating, body aches and itching	High lymphocyte	1	56	F	d
Severe anemia	High white blood cell and lymphocyte and low platelet	2	45	F	e
High white blood cell and low platelet	High white blood cell	1	49	M	f
High white blood cell	Low platelet	2	63	M	g
Body aches	High white blood cell and lymphocyte	2	55	M	h
Chest pain	High white blood cell and low platelet	2	62	F	i
High white blood cell and lymphocyte	High white blood cell and lymphocyte and low platelet	3	72	M	j
Excessive sweating	High lymphocyte	2	65	M	k
High lymphocyte	High white blood cell and lymphocyte	4 months	63	F	l
Low platelet	High lymphocyte and low platelet	1	52	M	m
Weakness	Low platelet	2	50	M	n
Low platelet and swelling of the lymph nodes	Low platelet	2	51	M	o
Anemia	High lymphocyte and low platelet	1.5	64	M	p
High lymphocyte	High lymphocyte	2	55	F	q
Weakness	Low platelet	2	66	M	r
High lymphocyte	High lymphocyte	3	60	M	s
Low platelet	Low platelet	2	66	M	t

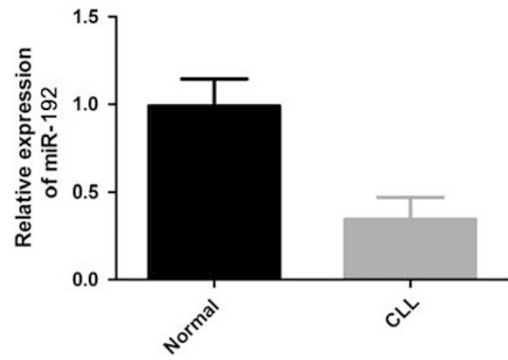


**Figure 1.** Condition of CBC in three patients and progression of the disease (a–c). CBC, complete blood count; RBC, red blood cell; PLT, platelets; WBC, white blood cell; WDF, WBC differential; WNR, white cell nucleated.

**Table 2.** Hematological parameters in three selected patients

Patient	Parameter	Count	Interval reference
A	RBC (cells per mL)	3.53	3.50–6.0
	HGB (g dl <sup>-1</sup> )	11.7	11.0–17.0
	HCT (%)	35.5	35.0–50.0
	MCV (fL)	100.6	80.0–100.0
	MCH (pg)	33.1	27.0–32.0
	MCHC (g dl <sup>-1</sup> )	33.0	31.0–36.0
	RDW–SD (fL)	48.8	37.0–54.0
	RDW–CV (%)	13.6	11.0–14.0
	PLT (mL)	124	150–450
	PDW (fL)	11.1	9.0–17.0
	MPV (fL)	10.4	9.0–13.0
	P–LCR (%)	28.3	13.0–43.0
	PCT (%)	0.13	0.17–0.35
	WBC (cells per mL)	12.67	4.0–10.0
	NEUT (cells per mL)	3.33	1.50–7.0
	LYMPH (cells per mL)	8.69	1.0–3.70
	MONO (cells per mL)	0.46	0.0–0.70
	EO (cells per mL)	0.16	0.0–0.40
	BASO (cells per mL)	0.03	0.0–0.10
	IG (mg dl <sup>-1</sup> )	0.07	0.00–7.0
B	RBC (cells per mL)	5.98	3.50–6.0
	HGB (g dl <sup>-1</sup> )	16.4	11.0–17.0
	HCT (%)	49.0	35.0–50.0
	MCV (fL)	81.9	80.0–100.0
	MCH (pg)	27.4	27.0–32.0
	MCHC (g dl <sup>-1</sup> )	33.5	31.0–36.0
	RDW–SD (fL)	39.8	37.0–54.0
	RDW–CV (%)	13.7	11.0–14.0
	PLT (mL)	136	150–450
	PDW (fL)	11.8	9.0–17.0
	MPV (fL)	11.6	9.0–13.0
	P–LCR (%)	39.4	13.0–43.0
	PCT (%)	0.16	0.17–0.35
	WBC (cells per mL)	98.48	4.0–10.0
	NEUT (cells per mL)	7.76	1.50–7.0
	LYMPH (cells per mL)	87.95	1.0–3.70
	MONO (cells per mL)	2.64	0.0–0.70
	EO (cells per mL)	0.06	0.0–0.40
	BASO (cells per mL)	0.07	0.0–0.10
	IG (mg dl <sup>-1</sup> )	0.24	0.00–7.0
C	RBC (cells per mL)	3.83	3.50–6.0
	HGB (g dl <sup>-1</sup> )	6.4	11.0–17.0
	HCT (%)	29.5	35.0–50.0
	MCV (fL)	77.0	80.0–100.0
	MCH (pg)	16.7	27.0–32.0
	MCHC (g dl <sup>-1</sup> )	21.7	31.0–36.0
	RDW–SD (fL)	50.3	37.0–54.0
	RDW–CV (%)	22.5	11.0–14.0
	PLT (mL)	133	150–450
	PDW (fL)	—	9.0–17.0
	MPV (fL)	—	9.0–13.0
	P–LCR (%)	—	13.0–43.0
	PCT (%)	—	0.17–0.35
	WBC (cells per mL)	451.55	4.0–10.0
	NEUT (cells per mL)	8.30	1.50–7.0
	LYMPH (cells per mL)	408.03	1.0–3.70
	MONO (cells per mL)	34.79	0.0–0.70
	EO (cells per mL)	0.34	0.0–0.40
	BASO (cells per mL)	0.39	0.0–0.10
	IG (mg dl <sup>-1</sup> )	2.14	0.00–7.0

Abbreviations: BASO, basophil; EO, eosinophil; IG, immunoglobulin; HCT, hematocrit; HGB, hemoglobin; LYMPH, lymphocytes; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MONO, monocytes; MPV, mean platelet volume; NEUT, neutrophils; PCT, procalcitonin; PDW, platelet distribution width; PLT, platelets; P–LCR, platelet large cell ratio; RBC, red blood cell; RDW–SD, red cell distribution width–s.d.; RDW–CV, red cell distribution width–coefficient of variation; WBC, white blood cell.



**Figure 2.** Comparison of RE CLL with control subjects. Downregulation of miR-192 in CLL patients. Quantitative PCR analysis of miR-192 expression level in PBMCs of CLL patients ( $n=20$ ) and normal controls ( $n=20$ ). Results are normalized to those of controls and are represented relative to expression of the small nuclear RNA U6. CLL, chronic lymphocytic leukemia; PBMCs, peripheral blood mononuclear cells.

**Table 3.** The seven most statistically relevant KEGG signaling pathways with miR-192 targetome (DAVID database)

Rank	KEGG pathway	The number of genes in the pathway	Statistical P-value
1	hsa: cell cycle	7	1.9E–3
2	hsa: prostate cancer	5	1.4E–2
3	hsa: pathways in cancer	9	2.1E–2
4	hsa: chronic myeloid leukemia	5	7.9E–3
5	hsa: small cell lung cancer	5	1.2E–2
6	hsa: ARVC	4	4.6E–2
7	has: dilated cardiomyopathy	4	7.3E–2

Abbreviation: ARVC, arrhythmogenic right ventricular cardiomyopathy.

according to the manufacturer's leaflet. Real-time quantitative PCR reactions were accomplished in triplicate. Briefly, in a total volume of 10  $\mu$ l, 20 ng  $\mu$ l<sup>-1</sup> of cDNA product was added to a master mix, including 10 pmol  $\mu$ l<sup>-1</sup> of miR-192 U6 (Housekeeping) primers (Pars genome) and 5 ml of SYBR premix ExTaq II (TaKaRa, Kusatsu, Shiga Prefecture, Japan). The run program was set at 95 °C for 5 min followed by 40 cycles of 95 °C for 5 s, 60 °C for 20 s and 72 °C for 30 s.

**Statistical analysis.** Real-time PCR data analysis was performed using the  $\Delta\Delta$ CT method and the final result was normalized by small nuclear RNA, U6, as an endogenous control. All statistical tests were performed using REST 2009 V2.0.13 and Graph Pad Prism statistical software, version 5.01 (Graph Pad, San Diego, CA, USA). For all tests a  $P$ -value of  $<0.05$  was considered statistically significant.

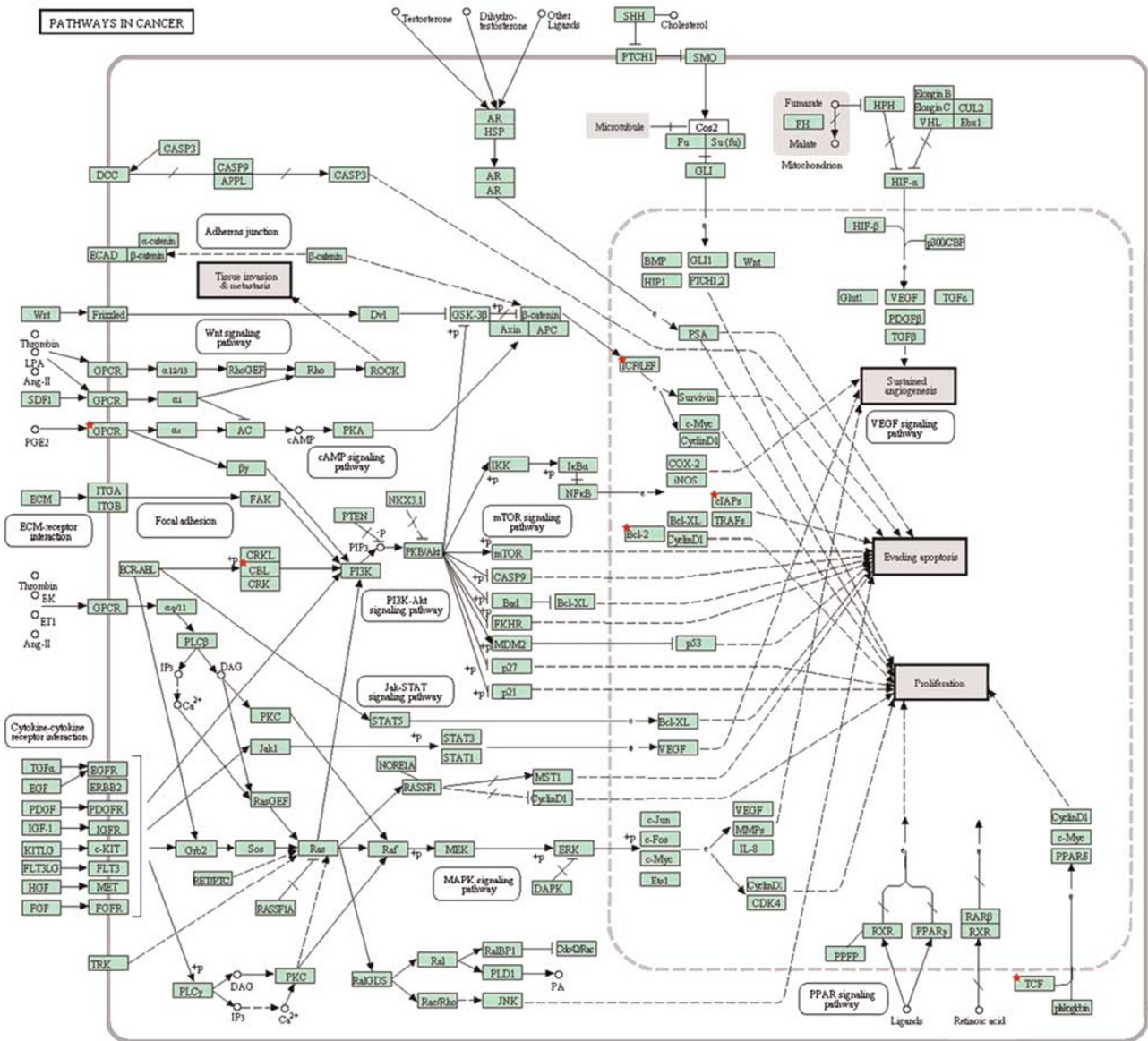
**Systematic pathway enrichment analysis.** In molecular enrichment analysis on miR-192 targetome and signaling pathways, several databases were used, including the miRecords<sup>22</sup> and miRTarBase<sup>23</sup> databases, to obtain predicted and validated targets of miR-192. Then, to fulfill signaling pathway enrichment analysis, miR-192 targetome was imputed in the database for annotation, visualization and integrated discovery (DAVID) online database, version 6.7.<sup>24</sup> This database provides the results from Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis to identify the signaling pathways and molecular networks with miR-192 targetome.<sup>25</sup>

## RESULTS

### miR-192 expression in CLL patients versus healthy subjects

The expression of miR-192 was evaluated by quantitative real-time PCR method in two groups: CLL patients ( $n=20$ ) and healthy subjects ( $n=20$ ). Clinical and biological features of patients are





**Figure 3.** Involvement of miR-192 targetome in cell cycle signaling pathway. For more information please see the KEGG database website: [http://www.genome.jp/dbget-bin/www\\_bget?map04350](http://www.genome.jp/dbget-bin/www_bget?map04350).

shown in Supplementary Table 1. Data analysis using SPSS statistical (Chicago, IL, USA) and REST software (Newcastle, UK) indicated a significant reduction in the expression of miR-192 in patients with CLL compared with healthy individuals ( $P = 0.002 \sim 2.5$ -fold; Figure 2).

In this study, three variables associated with CLL (that is, WBC, lymphocyte and platelet counts) were checked using SPSS software. White blood cell counts were higher than the reference interval in 65% of patients, also lymphocyte and platelet counts in 90% and 45% of patients, respectively.

**Molecular signaling pathway enrichment analysis**

Using miRecords and miRtarBase databases, 145 and 25 mRNAs were identified as validated and predicted targets of miR-192, respectively (Supplementary Table 1).

Predicted targets were approved by at least four prediction databases. All validated targets recovered from the miRtarBase

database were supported by strong experimental evidence such as reporter assay, western blot and quantitative real-time PCR. Imputing gene symbols of selected miR-192 targetome into a useful annotation tool of DAVID determined statistically significant organization of imputed genes with several KEGG signaling pathways (Table 3). The set of imputed genes as miR-192 targetome was mostly enriched in several KEGG pathways involved in cell cycle and cancer signaling (Table 3). The KEGG pathways in cancer signaling are shown in Figure 3 and targets of miR-192 are indicated by red stars.

**DISCUSSION**

In the present study, we showed a significant reduction in the expression of miR-192 in patients with CLL compared with healthy individuals. High expression of miR-192 inhibits metastatic colonization through increased apoptosis and decreased proliferation. CLL is defined as a disease of mature B cells. In CLL cancer cells

originate from lymphoid cells, particularly B lymphocytes. These immature lymphocytes are observed in the blood and bone marrow. The monoclonal B cells in CLL express CD19, CD5, and CD23. CLL is observed in lymph nodes, spleen and other tissues.<sup>2</sup> CLL leads to a decrease in platelet or erythrocyte count due to bleeding and bruising. Appropriate non-aggressive biomarkers with high sensitivity and specificity are required for the diagnosis of the disease. MiRNAs have emerged as biomarkers for the diagnosis and prognosis of different types of cancer.<sup>10,12,13,26</sup> Downregulation of miR-192 is correlated with the expression of Bcl-2, Zeb2 and VEGFA.<sup>17</sup> In addition, miR-192 expression is correlated with gastric cancer in distinct metastasis.<sup>18</sup> miR-192 can regulate p53, which enhances cyclin-dependent kinase inhibitor (CDKN1A/p21) and can adjust cell cycle arrest by inhibition of carcinogen. B-CLL cells are arrested in G0 (early G1) phase of the cell cycle that is controlled by cyclin d2 and d3 (cdk4). With stimulation of these cyclins, they could up-regulate.<sup>27</sup> PTEN protein was not expressed in 28% of CLL patients with a normal PTEN genotype that pointed to LOH at 10q23.3. Inhibition of the PI3K/Akt signaling pathway in CLL may play a therapeutic role in the absence of PTEN protein.<sup>28</sup> In addition, a decrease in PTEN increases PIP3 and Akt activation leading to the phosphorylation of Akt target proteins of miR-192. According to previous studies, B-cell lymphoma2 (BCL2) is a central player in promoting survival by inhibiting apoptosis and overexpression of Bcl2 in CLL.<sup>29</sup> On the other hand, accumulating studies have demonstrated that miR-192 suppresses Bcl-2, resulting in enhanced apoptosis.<sup>17</sup> Casitas B-lineage lymphoma (Cbl) is an E3 ubiquitin ligase of many tyrosine kinase receptors. The c-CBL and b-CBL genes are ubiquitously expressed with the highest levels in hematopoietic tissues.<sup>30</sup> Many studies have indicated that Cbl plays an important role in CLL. miR-192 is able to active BCR-ABL and PI3K by phosphorylation. This process shows that miR-192 must be downregulated in this disease. Peroxisome proliferators-activate receptor (PPAR)  $\alpha$  is a therapeutic target and a biological mediator of CLL. This pathway has been shown to activate proliferation in miR-192 by TCF.<sup>31</sup> It can be concluded from Figure 3 that miR-192 can suppress BCR-ABL, PI3K-Akt and PPAR signaling pathways, by targeting the main elements of this pathway including CBL, Bcl2, and TCF. These results suggest the relevance of miR-192 as a valuable biomarker in CLL patients. However, more evidence from loss- and gain-of-function variants is required to confirm this theory. It can be assumed that due to its reduction, miR-192 may have a tumor suppressing role in this disease. This microRNA could be a marker for early diagnosis of CLL.

## CONCLUSION

Despite achievements in monitoring of patients with CLL, an ideal biomarker for rapid and reliable diagnosis is still lacking. In this study, we investigated the transcript levels of miR-192 in CLL patients in comparison with normal subjects. We observed that the rate of miR-192 transcripts in CLL patients decreased. These observations proved that miR-192 can predict response to treatment. According to our results miR-192 could have an inducing role in CLL differentiation in possibly targeting some negative regulators of CLL differentiation. Our study of PBMCs in CLL patients showed downregulation in miR-192. This miRNA could be a potential marker for early diagnosis of CLL as well as a potential therapeutic target.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

This study was supported by Mansour Salehi (PhD) in Isfahan university of Medical Sciences. We thank all patients who contributed to this investigation.

## REFERENCES

- Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. *N Engl J Med* 2005; **352**: 804–815.
- Li S, Moffett HF, Lu J, Werner L, Zhang H, Ritz J *et al*. MicroRNA expression profiling identifies activated B cell status in chronic lymphocytic leukemia cells. *PLoS One* 2011; **6**: e16956.
- Lin K, Sherrington PD, Dennis M, Matrai Z, Cawley JC, Pettitt AR. Relationship between p53 dysfunction, CD38 expression, and IgV H mutation in chronic lymphocytic leukemia. *Blood* 2002; **100**: 1404–1409.
- Fernando TR, Rodriguez-Malave NI, Rao DS. MicroRNAs in B cell development and malignancy. *J Hematol Oncol* 2012; **5**: 7.
- Moussay E, Wang K, Cho J-H, van Moer K, Pierson S, Paggetti J *et al*. MicroRNA as biomarkers and regulators in B-cell chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2011; **108**: 6573–6578.
- Calin GA, Liu C-G, Sevignani C, Ferracin M, Felli N, Dumitru CD *et al*. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. *Proc Natl Acad Sci USA* 2004; **101**: 11755–11760.
- Teimori H, Ashoori S, Akbari MT, Naeini MM, Chaleshtori MH. FISH Analysis for del6q21 and del17p13 in B-cell chronic lymphocytic leukemia in Iranians. *Iran Red Crescent Med J* 2013; **15**: 107.
- Zent CS. Time to test CLL p53 function. *Blood* 2010; **115**: 4154–4155.
- Gholamin S, Pasdar A, Sadegh Khorrami M, Mirzaei H, Reza Mirzaei H, Salehi R *et al*. The potential for circulating microRNAs in the diagnosis of myocardial infarction: a novel approach to disease diagnosis and treatment. *Curr Pharm Des* 2016; **22**: 397–403.
- Mirzaei H, Gholamin S, Shahidsales S, Sahebkar A, Jafaari MR, Mirzaei HR *et al*. MicroRNAs as potential diagnostic and prognostic biomarkers in melanoma. *Eur J Cancer* 2016; **53**: 25–32.
- Simonian M, Mosallayi M, Mirzaei H. Circulating miR-21 as novel biomarker in gastric cancer: diagnostic and prognostic biomarker. *Cancer Res Ther* 2016.
- Reza Mirzaei H. Circulating microRNAs in hepatocellular carcinoma: potential diagnostic and prognostic biomarkers. *Curr Pharm Des* 2016 (e-pub ahead of print).
- Salarini R, Sahebkar A, Mirzaei H, Jaafari M, Riahi M, Hadjati J *et al*. Epi-drugs and Epi-miRs: moving beyond current cancer therapies. *Curr Cancer Drug Targets* 2015 (e-pub ahead of print).
- Bueno MJ, Malumbres M. MicroRNAs and the cell cycle. *Biochim Biophys Acta* 2011; **1812**: 592–601.
- Braun CJ, Zhang X, Savelyeva I, Wolff S, Moll UM, Schepeler T *et al*. p53-Responsive microRNAs 192 and 215 are capable of inducing cell cycle arrest. *Cancer Res* 2008; **68**: 10094–10104.
- Pettitt A, Sherrington P, Cawley J. The effect of p53 dysfunction on purine analogue cytotoxicity in chronic lymphocytic leukaemia. *Br J Haematol* 1999; **106**: 1049–1051.
- Geng L, Chaudhuri A, Talmon G, Wisecarver JL, Are C, Brattain M *et al*. MicroRNA-192 suppresses liver metastasis of colon cancer. *Oncogene* 2014; **33**: 5332–5340.
- Chen Q, Ge X, Zhang Y, Xia H, Yuan D, Tang Q *et al*. Plasma miR-122 and miR-192 as potential novel biomarkers for the early detection of distant metastasis of gastric cancer. *Oncol Rep* 2014; **31**: 1863–1870.
- Di Liso L, Sánchez-Beato M, Gómez-López G, Rodríguez ME, Montes-Moreno S, Mollejo M *et al*. MicroRNA signatures in B-cell lymphomas. *Blood Cancer J* 2012; **2**: e57.
- Georges SA, Biery MC, Kim S-y, Schelter JM, Guo J, Chang AN *et al*. Coordinated regulation of cell cycle transcripts by p53-Inducible microRNAs, miR-192 and miR-215. *Cancer Res* 2008; **68**: 10105–10112.
- Negrini M, Cutrona G, Bassi C, Fabris S, Zagatti B, Colombo M *et al*. microRNAome expression in chronic lymphocytic leukemia: comparison with normal B-cell subsets and correlations with prognostic and clinical parameters. *Clin Cancer Res* 2014; **20**: 4141–4153.
- Xiao F, Zuo Z, Cai G, Kang S, Gao X, Li T. miRecords: an integrated resource for microRNA-target interactions. *Nucleic Acids Res* 2009; **37**: D105–D110.
- Hsu S-D, Lin F-M, Wu W-Y, Liang C, Huang W-C, Chan W-L *et al*. miRTarBase: a database curates experimentally validated microRNA-target interactions. *Nucleic Acids Res* 2011; **39**: D163–169.
- Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2009; **4**: 44–57.
- Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 2000; **28**: 27–30.

- 26 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281–297.
- 27 Decker T, Schneller F, Miething C, Jahn T, Duyster J, Peschel C. Cell cycle progression of chronic lymphocytic leukemia cells is controlled by cyclin D2, cyclin D3, cyclin-dependent kinase (cdk) 4 and the cdk inhibitor p27. *Leukemia*. 08876924. 2002; **16**: 327–334.
- 28 Leupin N, Cenni B, Novak U, Hügli B, Graber HU, Tobler A *et al*. Disparate expression of the PTEN gene: a novel finding in B-cell chronic lymphocytic leukaemia (B-CLL). *Br J Haematol* 2003; **121**: 97–100.
- 29 Majid A, Tsoulakis O, Walewska R, Gesk S, Siebert R, Kennedy DBJ *et al*. BCL2 expression in chronic lymphocytic leukemia: lack of association with the BCL2 – 938 A> C promoter single nucleotide polymorphism. *Blood* 2008; **111**: 874–877.
- 30 McKeller MR, Robetorye RS, Dahia PL, Aguiar RC. Integrity of the CBL gene in mature B-cell malignancies. *Blood* 2009; **114**: 4321–4322.
- 31 Bernard P, Fleming A, Lacombe A, Harley VR, Vilain E. Wnt4 inhibits  $\beta$ -catenin/TCF signalling by redirecting  $\beta$ -catenin to the cell membrane. *Biol Cell* 2008; **100**: 167–177.

Supplementary Information accompanies the paper on Cancer Gene Therapy website (<http://www.nature.com/cgt>)