Jentashapir J Cell Mol Biol. 2021 March; 12(1):e112986. doi: 10.5812/jjcmb.112986.

Published online 2021 March 10. **Research Article**

Expression and Functional Assessment of Some Featured Coding and Non-coding RNAs Encoded by 8q24 Chromosomal Region in CML Patients

Mina Zamani [1], Hamid Galehdari [1,*], Babak Bakhshinejad [2], Mohammad-Reza Hajjari [1] and Ali-Mohammad Foroughmand [1]

[1]Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2]Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

[*]*Corresponding author*: Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran. Email: galehdari187@yahoo.com

Received 2021 January 14; **Revised** 2021 January 28; **Accepted** 2021 January 28.

Abstract

Background: The association between the human chromosomal 8q24 region and cancer development remains dim. The proto-oncogene *MYC* is known as the most prominent target of this chromosomal region. However, numerous cancer-associated genetic alterations in the region extend beyond the *MYC* locus. Accordingly, it is likely that the *MYC* oncogene is not the only target of these carcinogenesis-related alterations.

Objectives: In the present study, the expression of *MYC* and the correlation between *MYC* and two non-coding RNAs, namely *PVT1* (circular and linear forms) and *CASC11*, which are residents of the 8q24 region in the *MYC* neighborhood, were investigated in chronic myeloid leukemia (CML).

Methods: Real-time polymerase chain reaction (PCR) was used to assess *BCR-ABL* transcripts and categorize positive and negative (normal) samples for CML. Afterward, real-time PCR was exploited to evaluate the expression of different genes, including *MYC*, linear *PVT1*, circular *PVT* (*CircPVT1*), *CASC11*, and *ACTB* in CML and normal samples.

Results: We found that the expression of linear *PVT1* is significantly increased in CML compared with normal samples. However, *CircPVT1*, *CASC11*, and *MYC* did not show significantly altered expression between CML and normal groups. The experimental and in silico analyses of the correlation coefficients of gene expressions suggested changes in the correlations between the gene expressions in CML compared with normal samples. We also assessed the miR-trapping potential of *PVT1* and *CASC11* and the possible effects of these interactions on signaling pathways. Our findings indicated that these lncRNAs could have a possible regulatory link with critical pathways associated with leukemogenesis.

Conclusions: Our results indicate that non-coding genes surrounding *MYC* within the 8q24 region might have regulatory roles in CML carcinogenesis.

Keywords: CML, 8q24, *MYC*, Non-coding RNAs

1. Background

The Myc protein is a transcription factor with essential roles in cell growth and proliferation. Numerous genomic variations in the 8q24 region, including amplifications, translocations, breakpoints, viral integrations, and single nucleotide variations (SNVs) occur in the neighborhood regions of the *MYC* locus. Therefore, it is likely that *MYC* is not the only target of these cancer-associated alterations, and many other non-coding genes surrounding *MYC* might contribute to carcinogenesis (1, 2). In hematopoietic stem cells (HSCs) or multipotent progenitors, deregulated gene expression, which arises from chromosomal aberrations or epigenetic alterations, can result in a variety of hematopoietic malignancies (3). Over the recent years, in vitro and in vivo data have indicated that c-Myc is one of the pivotal transcription factors that play critical roles in regulating hematopoiesis (4). *MYC* deregulation is recurrently found in many types of human lymphomas and leukemias. Although *MYC* deregulation happens through translocation in Burkitt lymphoma and less frequently in other types of lymphoma, the *MYC* is frequently over-expressed in acute lymphoblastic and myeloid leukemia via mechanisms unrelated to chromosomal translocation. This increased expression is often associated with disease progression (4, 5). Chronic myeloid

leukemia (CML) is known to be driven by the *BCR-ABL1* fusion gene. After oncogenic translocation, the *BCR-ABL1* gene goes under the transcriptional control of the *BCR* promoter. However, the molecular mechanisms involved in the regulation of oncogene expression are mostly unknown. Myc is a well-recognized binding partner of *BCR*. *BCR-ABL1* and *BCR* are transcriptionally controlled by Myc (6, 7). In addition, the Myc protein plays a major role in *BCR-ABL1*-mediated transformation, mainly by acting as a cooperative oncogene with the fusion protein (8, 9).

Our current understanding of the underlying genetic basis of leukemia hinges on decades of protein-centered research, and the contribution of non-coding regions to the initiation, maintenance, and evolution of this disease remains to be revealed (10). Coding sequences account for less than 2% of the genome, and it has become uncovered that aberrations within the non-coding genome drive important cancer phenotypes. The extensive transcription of RNA from non-protein-coding regions is one of the substantial findings of the transcriptomics world (11). Long non-coding RNAs (lncRNAs) have attracted much attention in recent years as a new layer of gene regulation. They perform a variety of functions by interaction with DNA, RNA, and protein molecules, including modulators of mRNA processing, transcription and translation, a source of microRNAs and competing for endogenous RNAs (ceRNAs), and regulators of nuclear architecture as well as chromatin structure. A large body of emerging evidence has revealed a vital contribution of lncRNAs to cancer development and progression (12, 13).

2. Objectives

Even neighboring genes can have mutual effects in bi-directional or one-directional manners. *MYC* has been shown to be surrounded by different lncRNAs that might play roles as transcriptional regulators of this proto-oncogene (14, 15). Some non-coding genes are found to be located within 8q24, including plasmacytoma variant translocation 1 (*PVT1*), colon cancer-associated transcript (*CCAT*) family, prostate cancer-associated transcript (*PCAT*) family, to mention a few. These non-coding sequences have been studied in different cancer types (14, 16). However, their role in CML is obscure and remains to be explored. Here, we evaluated the expression correlation of *MYC* and its two adjacent non-coding RNAs; *PVT1* (located 51582 nucleotides downstream of *MYC*) in circular and linear forms and *CASC11* (located 1467 nucleotides upstream of *MYC*) in CML patients.

3. Methods

3.1. Sample Collection

Untreated cases included a total of 19 CML positive (CML samples) and 23 CML negative cases (normal samples), referring to the Narges Genetics and PND Laboratory, Ahvaz, Iran, from 2019-2020, who were enrolled in the present study. CML positive patients were clinically diagnosed to have leukemia with an increased number of white blood cells (WBCs) and molecularly assessed using *BCR-ABL* fusion transcript analysis.

3.2. RNA Extraction and cDNA Synthesis

10 mL of peripheral blood samples were taken from all cases and collected in the nuclease-free EDTA-containing tubes. The isolation of peripheral blood mononuclear cells (PBMCs) was conducted using Ficoll-Paque™ (Sigma-Aldrich, Germany), and total RNA was extracted using TRIzol® reagent (Thermo Fisher Scientific, USA). RNA concentration was measured by a NanoDrop™ 2000/c spectrophotometer (Thermo Fisher Scientific, USA) and stored at -80 °C. RNA integrity was assessed using electrophoresis on 1% agarose gel containing SafeStain (CinnaGen, Iran). RNA was treated with DNaseI (Takara Bio, Inc., Japan) and Prime-script™ RT reagent kit (Takara Bio, Inc., Japan) and was used for reverse transcription of RNA to cDNA.

3.3. Quantitative BCR-ABL Testing

BCR-ABL fusion gene transcript is detectable in more than 95% of CML patients. Also, these transcripts are rarely present in acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML) patients (this translocation is found in 10 to 20% of adults and in 2 to 5% of children with ALL, and in 1% approximately of AML cases). The level of *BCR-ABL/ABL* is lower (about 10 - 15%) in ALL and AML patients, while in most cases of CML, the range of *BCR-ABL/ABL* is 30 - 300% with an average of 80 - 90% (17). Quantification of the *BCR-ABL* fusion transcripts in both PBMCs and bone marrow aspirates of patients with CML using reverse transcription-polymerase chain reaction (RT-PCR) has been shown to be suitable for disease diagnosis and monitoring (18). Briefly, after cDNA synthesis, the number of fused transcripts produced from the Philadelphia chromosome was assessed using MBCR 210 RQ Kit (NovinGene, Iran) by quantitative RT-PCR. This kit is used for quantitative detection of BCR-ABL p210 translocation. Each sample was evaluated for the mRNA levels of the two transcripts; (p210) *ABL-BCR* and *ABL*. According to the five standards with definite copy numbers of *ABL-BCR* and *ABL*, the exact levels of these mRNAs were determined in the samples.

3.4. Gene Expression Analysis

The evaluation of gene expression was conducted on linear *PVT1*, circular *PVT1* (*CircPVT1*), *CASC11*, and *MYC* as target genes, and *ACTB* as an endogenous control gene. Primers were designed using AlleleID6.0 software. The list of primers for all targeted genes is indicated in Table 1. Real-time PCR was performed using the SYBR® Premix Ex-Taq TMII (Takara Bio Inc., Shiga, Japan). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

3.5. Statistical Analysis

The analysis of gene expression data was performed using a t-test by GraphPad Prism version 8 (GraphPad Software, Inc., USA). Spearman's rank correlation coefficient was used for correlation analysis of relative gene expression.

3.6. In silico Analysis

In silico analyses consist of two parts, including the assessment of gene expression correlation analysis and lncRNA/miR/mRNA network analysis.

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/detail.php?gene=&clicktag=survival) and GENT2 databases (http://gent2.appex.kr/gent2/) were used for gene expression correlation analysis.

The LncRNASNP tool (available via http://bioinfo.life.hust.edu.cn/lncRNASNP) was used to investigate the miRNA-trapping potential of lncRNAs. This bioinformatics tool enables us to search for miRNA binding sites on lncRNAs based on using different sources of data, including miRanda, TargetScan, and Pita. In the next step, miRTarBase, which includes all published miRNA target interaction data (http://mirtarbase.mbc.nctu.edu.tw/php/index.php), was used to predict the mRNA targets of previously found miRNAs. Then, SPEED (http://speed.sys-bio.net/) was applied to analyze signaling pathways in which these mRNAs are involved.

4. Results

4.1. BCR-ABL Translocation Analysis

BCR-ABL fused transcript is the diagnostic hallmark of CML. Accordingly, *BCR-ABL* transcript level was quantified in samples using the normalized copy number (NCN) method (19, 20). The ratio of *ABL-BCR* expression was normalized to *ABL* expression, and the percentage was calculated. According to the results, the samples were categorized as CML positive and negative (normal) (Table 2).

4.2. Gene Expression Analysis

The expression of *MYC*, linear *PVT1*, *CircPVT1*, and *CASC11*, which are located downstream and upstream of *MYC*, was evaluated in CML and normal groups. Gene expression analysis showed a significantly higher expression of linear *PVT1* ($P = 0.03$) in CML patients compared with normal samples. Our results indicated that the expression of the linear form of *PVT1*, but not a circular one, is remarkably enhanced in CML samples. *CASC11* and *MYC* also exhibited a trend of overexpression; however, it was not statistically significant (Figure 1).

4.3. Gene Expression Correlation Analysis

The correlation of gene expression in both CML positive and negative samples was assessed using Spearman's correlation coefficient (R) (Table 3). We did not find any statistically significant data for the correlation analyses ($P > 0.05$). However, it should be noticed that most of the correlations decreased in CML compared with the normal group. Because of our limited experimental samples, we also analyzed the in silico gene expression correlations data of *MYC* and its surrounding non-coding RNA genes, including *PVT1*, and *CASC11* to make more clear data. According to GEPIA and GENT2 analyses (Table 4), all gene expression (normalized to *ACTB*) correlations in the whole blood (TGEX) were moderately positive ($R > 0.5$, $P < 0.05$), whereas correlations were weak ($R < 0.3$, $P < 0.05$) in CML cell lines (TGEX) and CML samples (GENT2).

4.4. PVT1 and CASC11 / miRNA/ mRNA Potential Interaction

Our analyses suggested a collection of 94 miRs targeting *PVT1*. This collection of miRs targets 6750 coding genes. Protein-coding genes most significantly targeted by *PVT1*-trapped miRNAs are members of the TNFα signaling pathway (Figure 2). Also, our findings from lncRNASNP2 suggested that *CASC11* transcript harbors 87 miR binding sites. This bunch of miRs can target 5175 mRNAs of coding genes, most of which belong to the MAPK signaling pathway.

5. Discussion

The human chromosomal region 8q24, which is frequently disrupted in a variety of cancers, is the site for several lncRNAs in the neighborhood of *MYC* (21, 22). The aberrant expression of lncRNAs has been found to be associated with various human malignancies. However, limited data is available about the functional involvement of lncRNAs in CML tumorigenesis. In the present study, we performed a gene expression assessment for *MYC* and its two surrounding lncRNAs *CASC11* and *PVT1* (circular and linear

Table 1. Sequences of the Primers for Different Genes

Gene	Ref Seq (Assembly: GRCh38.p13)	Sequence
ACTB	NM_001101.5	
F		ATTGGCAATGAGCGGTTC
R		TGAAGGTAGTTTCGTGGATG
MYC	NM_002467.6; NM_001354870.1	
F		GCGACTCTGAGGAGGAAC
R		CTGCGTAGTTGTGCTGATG
PVT1	NR_003367.3	
F		CTTGGAGGCTGAGGAGTTCA
R		CTTCAGGCCTCTTTGACAGC
Circ-*PVT1*		
F		CGACTCTTCCTGGTGAAGCATCTGAT
R		TACTTGAACGAAGCTCCATGCAGC
CASC11	NR_117102.1; NR_117101.1	
F		GCAGAAGGTCCGAAGAAAGAG
R		TGTTCATTAGCAGTGGTGATAGG

Abbreviations: F, forward; R, reverse.

Table 2. Demographic and Clinical Characteristics of Enrolled Patients

Characteristics	Mean (Min-Max)
Age (y)	44.6 ± 13.9 (28-76)
Gender (M/F)	10/9
White blood cell count (10^9/ L)	330 ± 235 (88 - 714)
(BCR-ABL)/ABL%	77.53 ± 0.83 (13.95 - 206.13)

forms), which are located within the 8q24 region. Our results highlighted that *PVT1* linear RNA has a significantly higher expression (P = 0.0317) in CML patients compared with the normal group. *PVT1* has been shown to be dysregulated in several cancers. Also, recently, the elevation of this lncRNA has been reported in some kinds of leukemia, including APL and AML (23, 24). Here, we revealed that this lncRNA is upregulated in CML. It seems that *PVT1* linear RNA represents the potential to be used as a marker for CML detection. *CASC11* has been studied recently and identified as an oncogenic lncRNA (25-27). *CASC11* locus and transcript encompass the rs16902359 SNP, which showed an association with lymphoma (28). However, there is no report about this lncRNA in leukemia and the present study is the first report regarding the *CASC11* assessment in leukemia. Although we did not observe significant changes in *CASC11* expression, the average increase in the expression of this gene can indicate that there might be a significant increase merely in a subgroup of CML samples. Further studies with more samples and sub classified of the disease can provide more accurate information.

MYC also showed a trend of overexpression, but it was not statistically significant. Some reports showed significant overexpression of *MYC* in advanced phases of CML (29, 30). We did not categorize CML subtypes; thus, the lack of significant overexpression of *MYC* could be because of the presence of different types of CML in our study.

Neighboring genes can affect each other in bi-directional or one-directional manners. This effect is highly pronounced in the immediate vicinity (< 100 kb), but it can extend much further (31). Cis-acting lncRNAs have been demonstrated to activate, repress, or otherwise modulate the expression of target genes through various mechanisms (13). *PVT1* is a cis-regulatory lncRNA that harbors numerous enhancer elements (21, 32). To find a possible relationship between *MYC*, *PVT1*, and *CASC11*, we analyzed the correlation between the expression of genes. According to the experimental and in silico results, it seems that the target gene expression correlations mostly decreased in CML compared with the normal group. In cancer cells, distributions of chromatin folding and gene regulation occur in various tumor types, and certain regions are affected in a cancer-specific manner (33). It seems the moderate positive correlations between target genes in normal samples impair in CML, which is likely due to the epigenetics alteration changes in chromatin folding, and accessibility of enhancers in genomic regions

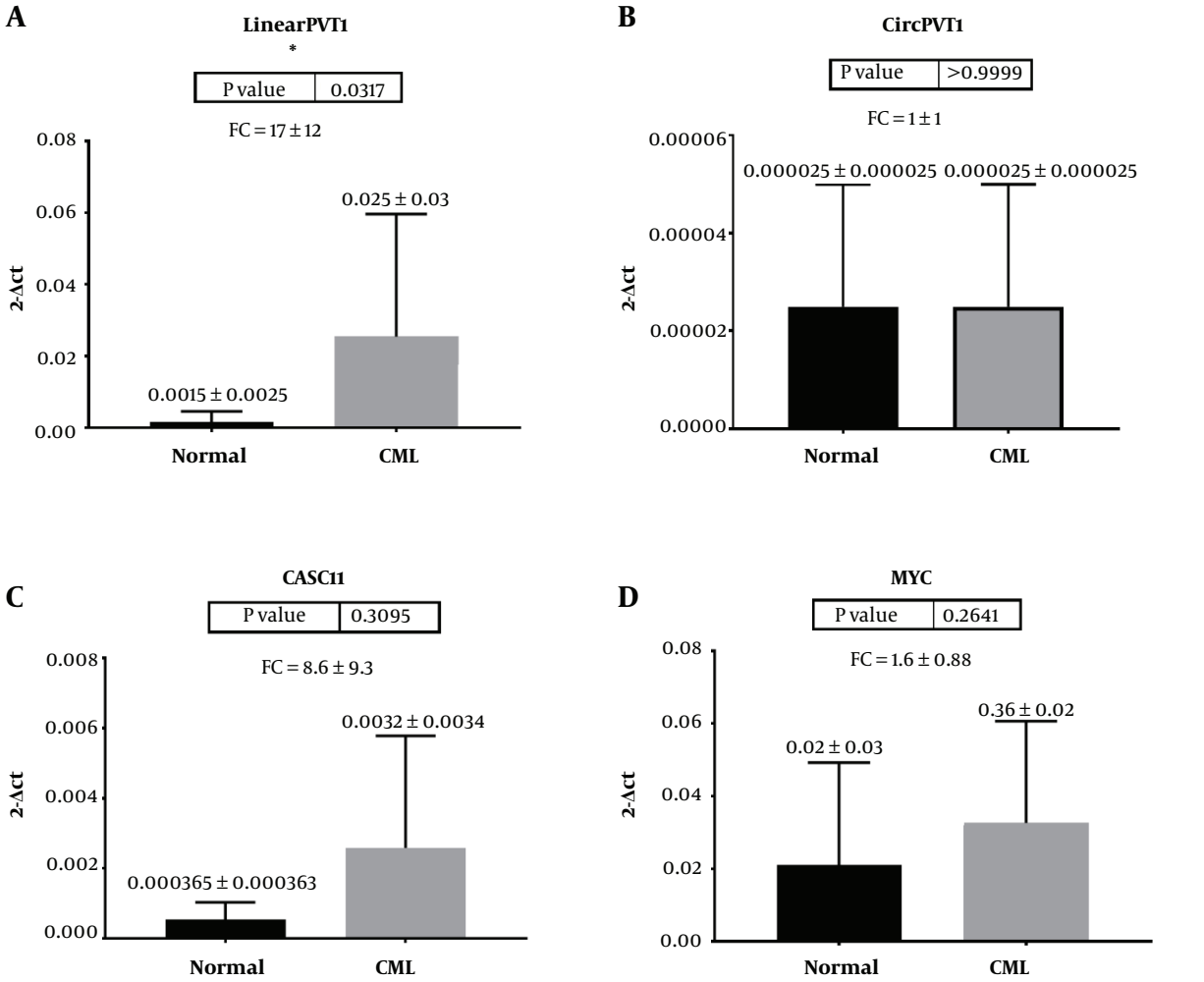


Figure 1. Gene expression analysis in chronic myeloid leukemia (CML) and normal samples. a) Linear *PVT1*, b) Circular *PVT1*, c) *CASC11*, and d) *MYC*. Data indicate a significant overexpression of Linear *PVT1* in CML compared with the normal group. (P-values below 0.05 were considered statistically significant, fold change (FC) = $2^{-\Delta\Delta Ct}$)

in cancerous compared with the normal status.

We also assessed the miR-sponging potency of target genes and the possible effect of these interactions on signaling pathways. Carcinogenesis-related signaling pathways could be regulated by *PVT1* and *CASC11* through the RNA network (Figure 2). TNFα is highly detected in CML patients and may serve as a new target therapy for the disease (34). *PVT1* overexpression in CML samples with subsequently increased potential of miRNA trapping can be accompanied by the availability of the members of these pathways, in particular, TNFα and promotion of leukemogenesis. Therefore, we suggest *PVT1* as a potential enhancer of the TNFα pathway via sponging miRs. Notably, Tang et al. recently reported regulation of TNFα by *PVT1* in rheumatoid arthritis (35). Further experimental studies could shed more light on our hypothesis.

Altogether, our findings based on targeted coding and non-coding RNAs in 8q24 expression analysis highlight the significant over-expression of linear-*PVT1* in CML samples compared with normal ones. Our results imply that *MYC*, *PVT*, and *CASC11* correlations are decreased in CML cancerogenesis; however, increasing the experimental sample size could be an effective way to obtain more accurate results. These transcripts could potentially affect some critical CML-related signaling pathways through the lncRNA-miR-mRNA network. Using knockout or knockdown mod-

Table 3. Gene Expression Correlation Analysis of Coding and Non-coding RNAs from 8q24

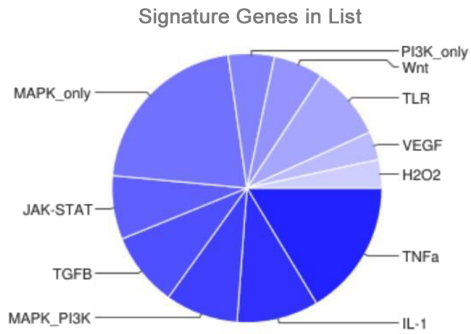
Genes (normalized to ACTB)	Normal		CML	
	R	P-Value	R	P-Value
MYC- Linear *PVT1*	- 0.30	0.68	- 0.60	0.35
MYC-CASC11	0.20	0.91	0.20	0.08
Linear *PVT1-CASC11*	0.90	0.08	-0.80	0.78
CircPVT1- MYC	-0.50	0.91	-1	0.08
CircPVT1- Linear *PVT1*	-0.80	0.08	0.20	0.91
CircPVT1- CASC11	-0.80	0.33	-0.40	0.75

Table 4. Gene Expression Correlation Analysis of Coding and Non-coding RNAs from 8q24 (In silico)

Genes (Normalized to ACTB)	Whole Blood (GTEX)		CML Cell Line (GTEX)		CML Samples (GENT2)	
	R	P-Value	R	P-value	R	P-Value
MYC-PVT1	0.64	0	0.17	0.17	0.49	1.58e-006
MYC-CASC11	0.56	0	0.54	2.2e-06	- 0.03	0.74
PVT1-CASC11	0.61	0	0.11	0.36	- 0.15	0.16

A

Pathway	Genes in List	Genes in Background	P-Value	FDR
TNFa	77	259	4.76e-21	2.87e-20
IL-1	46	141	8.48e-15	2.45e-14
MAPK_PI3K	41	118	2.13e-14	3.86e-14
TGFB	42	142	4.9e-12	9.05e-12
JAK-STAT	36	114	1.99e-11	2.43e-11
MAPK_only	100	559	8.18e-11	7.04e-11
PI3K_only	26	67	7.05e-11	7.46e-11
Wnt	28	83	6.08e-10	5.1e-10
TLR	42	181	1.74e-08	1.57e-08
VEGF	16	56	3.11e-05	2.19e-05
H2O2	16	60	7.84e-05	4.72e-05



B

Pathway	Genes in List	Genes in Background	P-Value	FDR
MAPK_only	94	559	7.05e-09	4.73e-08
PI3K_only	22	67	6.87e-08	2.86e-07
MAPK_PI3K	28	118	2.38e-06	4.11e-06
TNFa	48	259	2.23e-06	4.93e-06
VEGF	16	56	3.02e-05	4.43e-05
Wnt	19	83	0.000159	0.000191
H2O2	15	60	0.000275	0.000317
IL-1	25	141	0.00109	0.00107
TGFB	23	142	0.00549	0.00502
JAK-STAT	19	114	0.00809	0.00648
TLR	23	181	0.0719	0.0551

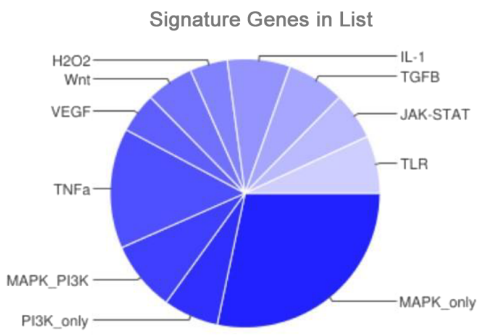


Figure 2. Potential signaling pathways in regulatory link with a) *PVT1*, and b) *CASC11*. TNFα and MAPK signaling pathways are the most significant pathways that are related respectively to *PVT1* and *CASC11* through the lncRNA/miRs/mRNAs network.

els of these transcripts, as well as the assessment of the activity and expression of members of introduced signaling pathways, our preliminary findings can be approved.

Acknowledgments

We gratefully acknowledge the cooperation of the patients who made this work possible. We would like to thank Prof. Dr. Rory Johnson for his help. We also appreciate all the staff of the Narges Medical Genetics and Prenatal Diagnosis Laboratory for their assistance.

Footnotes

Authors' Contribution: MZ and HG conceived and designed the study; MZ acquisition and analysis of the data; HG, AMF, and MRH supervised the research. MZ and BB drafted and revised the manuscript.

Conflict of Interests: The authors have no conflict of interest to declare.

Ethical Approval: The present study was approved by the Ethics Committee of the Shahid Chamran University of Ahvaz, Ahvaz, Iran (Ethics code: EE/99.3.02.65802/scu.ac.ir).

Funding/Support: The present study was funded by Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Informed Consent: Informed consent was obtained from the enrolled patients.

References

1. Grisanzio C, Freedman ML. Chromosome 8q24-Associated Cancers and MYC. *Genes Cancer*. 2010;**1**(6):555–9. doi: 10.1177/1947601910381380. [PubMed: 21779458]. [PubMed Central: PMC3092220].
2. Jin DH, Park SE, Lee J, Kim KM, Kim S, Kim D, et al. Copy Number Gains at 8q24 and 20q11-q13 in gastric cancer are more common in intestinal-type than diffuse-type. *PLoS One*. 2015;**10**(9). e0137657. doi: 10.1371/journal.pone.0137657. [PubMed: 26360582]. [PubMed Central: PMC4567330].
3. Vundinti BR, Ghosh K. Chromosomal aberrations in hematological malignancies: A guide to the identification of novel oncogenes. *Indian J Hum Genet*. 2011;**17**(2):43–4. doi: 10.4103/0971-6866.86168. [PubMed: 22090710]. [PubMed Central: PMC3214315].
4. Delgado MD, Leon J. Myc roles in hematopoiesis and leukemia. *Genes Cancer*. 2010;**1**(6):605–16. doi: 10.1177/1947601910377495. [PubMed: 21779460]. [PubMed Central: PMC3092227].
5. Kawagoe H, Kandilci A, Kranenburg TA, Grosveld GC. Overexpression of N-Myc rapidly causes acute myeloid leukemia in mice. *Cancer Res*. 2007;**67**(22):10677–85. doi: 10.1158/0008-5472.CAN-07-1118. [PubMed: 18006809].
6. Mahon GM, Wang Y, Korus M, Kostenko E, Cheng L, Sun T, et al. The c-Myc Oncoprotein Interacts with Bcr. *Curr Biol*. 2003;**13**(5):437–41. doi: 10.1016/s0960-9822(03)00090-3.
7. Sharma N, Magistroni V, Piazza R, Citterio S, Mezzatesta C, Khandelwal P, et al. BCR/ABL1 and BCR are under the transcriptional control of the MYC oncogene. *Mol Cancer*. 2015;**14**(1):132. doi: 10.1186/s12943-015-0407-0. [PubMed: 26179066]. [PubMed Central: PMC4504180].
8. Gopal V, Kadam P, Preisler H, Hulette B, Li YQ, Steele P, et al. Abnormal regulation of the myc gene in myeloid leukemia. *Med Oncol Tumor Pharmacother*. 1992;**9**(3):139–47. doi: 10.1007/BF02987745. [PubMed: 1341325].
9. Sawyers CL, Callahan W, Witte ON. Dominant negative MYC blocks transformation by ABL oncogenes. *Cell*. 1992;**70**(6):901–10. doi: 10.1016/0092-8674(92)90241-4. [PubMed: 1525828].
10. Ng M, Heckl D, Klusmann JH. The regulatory roles of long noncoding RNAs in acute myeloid leukemia. *Front Oncol*. 2019;**9**:570. doi: 10.3389/fonc.2019.00570. [PubMed: 31338324]. [PubMed Central: PMC6629768].
11. Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. *Cancer Cell*. 2016;**29**(4):452–63. doi: 10.1016/j.ccell.2016.03.010. [PubMed: 27070700]. [PubMed Central: PMC4831138].
12. Yousefi H, Maheronnaghsh M, Molaei F, Mashouri L, Reza Aref A, Momeny M, et al. Long noncoding RNAs and exosomal lncRNAs: classification, and mechanisms in breast cancer metastasis and drug resistance. *Oncogene*. 2020;**39**(5):953–74. doi: 10.1038/s41388-019-1040-y. [PubMed: 31601996].
13. Gil N, Ulitsky I. Regulation of gene expression by cis-acting long noncoding RNAs. *Nat Rev Genet*. 2020;**21**(2):102–17. doi: 10.1038/s41576-019-0184-5. [PubMed: 31729473].
14. Iaccarino I. lncRNAs and MYC: An intricate relationship. *Int J Mol Sci*. 2017;**18**(7). doi: 10.3390/ijms18071497. [PubMed: 28704924]. [PubMed Central: PMC5535987].
15. Marchese FP, Raimondi I, Huarte M. The multidimensional mechanisms of long noncoding RNA function. *Genome Biol*. 2017;**18**(1):206. doi: 10.1186/s13059-017-1348-2. [PubMed: 29084573]. [PubMed Central: PMC5663108].
16. Derderian C, Orunmuyi AT, Olapade-Olaopa EO, Ogunwobi OO. PVT1 signaling is a mediator of cancer progression. *Front Oncol*. 2019;**9**:502. doi: 10.3389/fonc.2019.00502. [PubMed: 31249809]. [PubMed Central: PMC6582247].
17. Hamid M, Bokharaei H. The frequency of BCR-ABL1 fusion transcripts in iranian patients with three different types of leukemia. *Zahedan J Res Med Sci*. 2017;**19**(7). doi: 10.5812/zjrms.10197.
18. Hochhaus A, Weisser A, La Rosee P, Emig M, Muller MC, et al. Detection and quantification of residual disease in chronic myelogenous leukemia. *Leukemia*. 2000;**14**(6):998–1005. doi: 10.1038/sj.leu.2401811. [PubMed: 10865964].
19. Gabert J, Beillard E, van der Velden VH, Bi W, Grimwade D, Pallisgaard N, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a europe against cancer program. *Leukemia*. 2003;**17**(12):2318–57. doi: 10.1038/sj.leu.2403135. [PubMed: 14562125].
20. Beillard E, Pallisgaard N, van der Velden VH, Bi W, Dee R, van der Schoot E, et al. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) - a Europe against cancer program. *Leukemia*. 2003;**17**(12):2474–86. doi: 10.1038/sj.leu.2403136. [PubMed: 14562124].
21. Huppi K, Pitt JJ, Wahlberg BM, Caplen NJ. The 8q24 gene desert: an oasis of non-coding transcriptional activity. *Front Genet*. 2012;**3**:69. doi: 10.3389/fgene.2012.00069. [PubMed: 22558003]. [PubMed Central: PMC3339310].
22. Hirano T, Yoshikawa R, Harada H, Harada Y, Ishida A, Yamazaki T. Long noncoding RNA, CCDC26, controls myeloid leukemia cell growth through regulation of KIT expression. *Mol Cancer*. 2015;**14**:90. doi: 10.1186/s12943-015-0364-7. [PubMed: 25928165]. [PubMed Central: PMC4423487].
23. Ghadiri A, Sharifi M, Mehrzad V, Bagheri P. Reduce proliferation of human bone marrow cells from acute myeloblastic leukemia with minimally differentiation by blocking lncRNA PVT1. *Clin Transl Oncol*. 2020;**22**(11):2103–10. doi: 10.1007/s12094-020-02360-4. [PubMed: 32406010].

24. Zeng C, Yu X, Lai J, Yang L, Chen S, Li Y. Overexpression of the long non-coding RNA PVT1 is correlated with leukemic cell proliferation in acute promyelocytic leukemia. *J Hematol Oncol*. 2015;**8**:126. doi: 10.1186/s13045-015-0223-4. [PubMed: 26545364]. [PubMed Central: PMC4636781].
25. Han Y, Chen M, Wang A, Fan X. STAT3-induced upregulation of lncRNA CASC11 promotes the cell migration, invasion and epithelial-mesenchymal transition in hepatocellular carcinoma by epigenetically silencing PTEN and activating PI3K/AKT signaling pathway. *Biochem Biophys Res Commun*. 2019;**508**(2):472–9. doi: 10.1016/j.bbrc.2018.11.092. [PubMed: 30503497].
26. Zhang Z, Zhou C, Chang Y, Zhang Z, Hu Y, Zhang F, et al. Long non-coding RNA CASC11 interacts with hnRNP-K and activates the WNT/β-catenin pathway to promote growth and metastasis in colorectal cancer. *Cancer Letters*. 2016;**376**(1):62–73. doi: 10.1016/j.canlet.2016.03.022.
27. Zhang L, Kang W, Lu X, Ma S, Dong L, Zou B. LncRNA CASC11 promoted gastric cancer cell proliferation, migration and invasion in vitro by regulating cell cycle pathway. *Cell Cycle*. 2018;**17**(15):1886–900. doi: 10.1080/15384101.2018.1502574. [PubMed: 30200804]. [PubMed Central: PMC6152531].
28. Morton LM, Purdue MP, Zheng T, Wang SS, Armstrong B, et al. Risk of non-Hodgkin lymphoma associated with germline variation in genes that regulate the cell cycle, apoptosis, and lymphocyte development. *Cancer Epidemiol Biomarkers Prev*. 2009;**18**(4):1259–70. doi: 10.1158/1055-9965.EPI-08-1037. [PubMed: 19336552]. [PubMed Central: PMC2716008].
29. Chandran RK, Geetha N, Sakthivel KM, Kumar RS, Krishna K, Sreedharan H. Differential gene expression changes and their implication on the disease progression in patients with Chronic Myeloid Leukemia. *Blood Cells Mol Dis*. 2019;**77**:51–60. doi: 10.1016/j.bcmd.2019.03.004. [PubMed: 30959263].
30. Beck Z, Bacsi A, Kovacs E, Kiss J, Kiss A, Balogh E, et al. Changes in oncogene expression implicated in evolution of chronic granulocytic leukemia from its chronic phase to acceleration. *Leuk Lymphoma*. 1998;**30**(3-4):293–306. doi: 10.3109/10428199809057542. [PubMed: 9713961].
31. Ghanbarian AT, Hurst LD. Neighboring genes show correlated evolution in gene expression. *Mol Biol Evol*. 2015;**32**(7):1748–66. doi: 10.1093/molbev/msv053. [PubMed: 25743543]. [PubMed Central: PMC4476153].
32. Cho SW, Xu J, Sun R, Mumbach MR, Carter AC, Chen YG, et al. Promoter of lncRNA gene PVT1 Is a tumor-suppressor DNA boundary element. *Cell*. 2018;**173**(6):1398–1412 e22. doi: 10.1016/j.cell.2018.03.068. [PubMed: 29731168]. [PubMed Central: PMC5984165].
33. Akdemir KC, Le VT, Chandran S, Li Y, Verhaak RG, Beroukhim R, et al. Disruption of chromatin folding domains by somatic genomic rearrangements in human cancer. *Nat Genet*. 2020;**52**(3):294–305. doi: 10.1038/s41588-019-0564-y. [PubMed: 32024999]. [PubMed Central: PMC7058537].
34. Shen N, Liu S, Cui J, Li Q, You Y, Zhong Z, et al. Tumor necrosis factor alpha knockout impaired tumorigenesis in chronic myeloid leukemia cells partly by metabolism modification and miRNA regulation. *Onco Targets Ther*. 2019;**12**:2355–64. doi: 10.2147/OTT.S197535. [PubMed: 31015764]. [PubMed Central: PMC6446984].
35. Tang J, Yi S, Liu Y. Long non-coding RNA PVT1 can regulate the proliferation and inflammatory responses of rheumatoid arthritis fibroblast-like synoviocytes by targeting microRNA-145-5p. *Hum Cell*. 2020;**33**(4):1081–90. doi: 10.1007/s13577-020-00419-6. [PubMed: 32918701].