Research Article

The Comparative Analysis of Gene Expression Profiles in Lymph Node Cells of Naturally BLV-infected and Uninfected Bovine

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Abstract

Bovine leukemia virus (BLV) is the etiologic agent of enzootic bovine leucosis (EBL) for the bovine host. In this study to examine gene expression changes in the manifestation of the EBL malignancy, four pooled RNA samples (three RNAs in each sample) were applied for transcriptome sequencing using RNA-seq technique. Differential expression analysis was done to compare the infected bovine group with the healthy bovine group using DESeq2 package in R software. Furthermore, functional gene ontology (GO) term and KEGG pathway enrichment analysis were stablished using the DAVID online database to identify involved GO terms and pathways in the host response to BLV infection. Our results suggested that 371 up- and 72 downregulated genes were involved in EBL with statistically significant threshold log2foldchange (LFC) = 1 and false discovery rate (FDR) <0.05 that were enriched in 74 biological processes and 20 KEGG pathways. Most of identified genes were associated with cancer, especially B-cell malignancies. The glycolysis/glycogenesis metabolic process is activated in B cells that confers growth and survival advantages in tumor and dysregulated CXCL10, IL17R, BTK, CDK4 and SYK genes known as valid biomarkers to increase the proliferation of malignant cell. The outcomes can provide a list of involved genes in the malignancy and help to screen candidate genes for cancer therapy in the future.

***Keywords*:** BLV, EBL, transcriptome, RNA-Seq, Gene Ontology

Introduction

Bovine leukemia virus, a deltaretrovirus related to human T-cell leukemia virus types 1 and 2 (HTLV-1 and HTLV-2), causes a lethal lymphoma or lymphosarcoma, enzootic bovine leucosis, which has been defined as a prolonged course that often involves persistent lymphocytosis (PL) and ends in B-cell lymphoma (Burny et al., 1988; Juliarena et al., 2017). EBL happens in a small portion (< 5-10%) of infected adult bovine, while the majority of infected animals (~70%) remain asymptomatic carriers. The prevalence of BLV infection and inducing of EBL in carriers cause economic losses for the livestock industry in the worldwide (Polat et al., 2017) and this infection is as high as 25.4% in the northeast of Iran (Mousavi et al., 2014).

The BLV virus carries two single-strand RNA (ssRNA), which can be reverse transcribed into DNA and integrated into the host genome as a proviral DNA (Moratorio et al., 2013). It encodes a trans-activator protein Tax, which plays a leading role in viral replication and activates dysregulation of cellular genes including cytokines, adhesion molecules and growth factors at the initial steps of oncogenesis to affect viral spreading and disease progression (Arainga et al., 2012). Furthermore, Tax gene can transcriptionally change the expression level of cellular genes involved in the cell cycle, DNA repair and programmed cell death processes (Aida et al., 2013; Arainga et al., 2012; Kouznetsova et al., 2019) to provide the basis of cell transformation. Despite the activation of Tax protein, the expression of antisense transcripts AS1 and AS2 from 3'LTR side of provirus have pivotal roles in the maintenance of malignancy and escaping from host immune responses, mainly at the latent stage of malignancy (Durkin et al., 2016).

The viral infection and tax-responsive genes might not be unique supplier for the cell transformation while other additional events are required such as gene polymorphisms, chromosomal aberrations, genome instability and accumulation of mutations in cellular DNA (Klener et al., 2006) for progression of this multistep tumor in the bovine host. The existence of polymorphism in the enhancer region of TNF-α (Konnai et al., 2006) and mutation in

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oncogenes such as *P53* (Dequiedt et al., 1995) is confirmed to contribute in the progression of BLV-induced lymphoma. Therefore, the virus-host interaction manner apart from viral factors would have a principal role to induce malignancy.

The objective of this study was to investigate the gene expression changes in the host-virus interaction manner using RNA-seq technique. It is known a little about the transcriptional changes associated with abnormal B-cell growth in EBL malignancy. We compared gene expression profiles of EBL and the healthy bovine groups to identify differentially expressed genes (DEGs). Subsequently, the identified DEGs were enriched to determine functional GO terms and KEGG pathways that participate in the B-cell transformation and EBL oncogenicity.

Materials and Methods

Sample collection and EBL diagnostics

This study was approved and supervised by the ethics committee of Ferdowsi University of Mashhad, Mashhad, Iran (No. 42333). Six infected lymphoid tumors at the acute stage of the EBL disease, and six healthy lymph nodes without BLV viral infection collected from cows (3.5-4 years old), at the slaughterhouse of Mashhad, Iran, and immediately transferred to a liquid nitrogen tank. At first, the EBL disease approved by two veterinarians based on the existence of clinical symptoms. Then, a qualitative method was optimized using the *Pol* gene expression in Rotor-gene 6000 (Qiagen, Germany) machine. Reaction ingredients were composed of 10 µl commercial Real-time master mix (Thermo Fisher, USA), 0.5 µl of primers (Fw, 5'-CCTCAATTCCCTTTAAACTA-3'; Rv, 5'-GTACCGGGAAGACTGGATTA-3'), 1 µL of TaqMan probe (FAM-GAACGCCTCCAGGCCCTTCA-BHQ1) (Rola-Łuszczak et al., 2013), 2.5 µl of deionized water and 5 µl of template DNA. DNAs were extracted with high quality by using the High Pure PCR template Kit (Qiagen, Germany), according to the manufacturer's instruction.

RNA extraction and sequencing

Total RNAs were isolated using the RNeasy Mini Kit (Qiagen, Germany), according to the manufacturer's instructions. The pooled RNAs per group (infected and uninfected) were prepared by mixing equal concentrations of three biological replicates (RNAs) to generate four pooled RNA samples. The single-strand DNA (ssDNA) templates were synthesized using oligo-dT primers and reverse transcriptase, according to the manufacturer's instructions (Thermo Fisher, USA). The purity, integrity and quantity of RNAs and cDNAs with amplification of the GAPDH gene (as a reference gene) were examined on 1.5% agarose gel and NanoDrop device. The type of poly-A capturing library was used for sequencing with Illumina HiSeq 4000 platform to generate 2-100 bp paired-end reads, and 2-30 million raw paired-end read files, by the BGI Company.

Differential expression analysis

Raw reads were preprocessed using FastQC (Andrews, 2016) and Trimmomatic (Bolger et al., 2014) software; then clean reads were acquired for subsequent analyses. The clean raw paired-end read profiles from each sample were mapped to the host-provirus reference genome individually, using STAR software (v2.5.4) (Dobin et al., 2013). Host-provirus reference genome was built by merging bovine reference genome UMD3.1 with proviral genome sequence BLV-YR2 (GenBank: KT122858) before alignment. Sorting and indexing of STAR outputs were performed by SAMtools software (v1.9) (Li et al., 2009). Annotation of GTF files (v74) for bovine, was downloaded from Ensemble database, and custom BLV virus annotation was built by HTLV-1/BLV genomics lab in Medical Genomics/Unit of Animal Genomics (UAG), Liege, Belgium. The FeacureCounts package was used for reads quantification (Liao et al., 2013). Finally, differential expression analysis was performed based on statistically significant FDR $\leq$ 0.05 in R software (v.3.5), using DESeq2 package (v.1.20) (Love et al., 2014).

Gene ontology (GO) and pathway analysis

Gene ontology analysis was performed on the list of significant (DEGs) based on biological process (BP), molecular function (MF), cellular component (CC) terms and the KEGG (The Kyoto Encyclopedia of Genes and Genomes) pathway, using DAVID 6.8 web tools (https://david.ncifcrf.gov/) (Huang et al., 2009). For identification of related GO terms to our samples, the GO profiles of significant DEGs were compared to the GO profile of bovine genome annotation as a reference set. The FDR $\leq$ 0.05 and P-value $\leq$ 0.01 were considered as a cutoff threshold for statistical significance in detection of GO terms and the KEGG pathways, respectively. The identified GO terms were classified using CateGOrizer "GO term classification counter" online tools based on GO slim method (Hu et al., 2008).

Results

EBL diagnostics

The Enzootic bovine leucosis can be characterized by developing tumor masses, tumor masses which are composed of monomorphic lymphocytic cells, and variable clinical signs including cardiac lymphosarcoma, enlarged lymph nodes and discrete nodular masses or a diffuse tissue infiltrate in EBL samples that were shown in Figure 1. The amplification of *Pol* gene by real-time PCR technique confirmed the infection of EBL samples, while the healthy subjects were not seropositive for BLV infection (Figure 2).

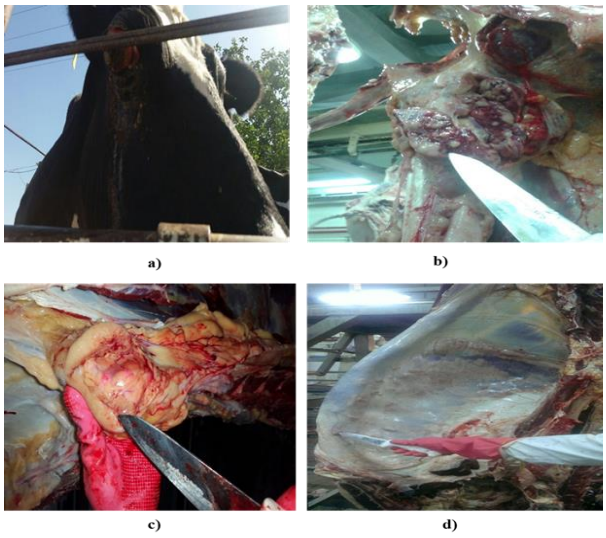

Figure 1. The clinical signs of EBL samples. a) Retro-ocular lymph node b) Cardiac lymphosarcoma, c) Enlarged mediastinal lymph node and d) Nodular masses.

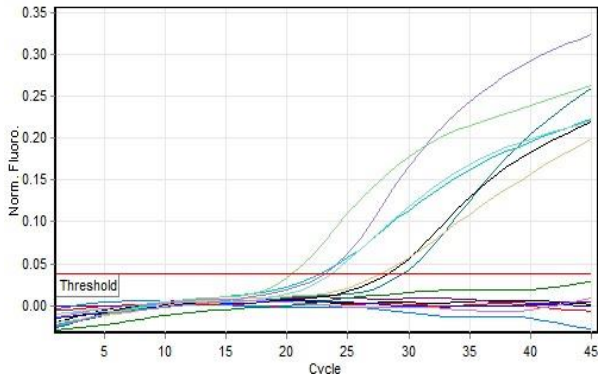

Figure 2. RT-qPCR amplification curves of *Pol* gene in healthy and infected samples.

RNA extraction and preprocessing of raw reads

The appearance of 28S and 18S ribosomal RNA and amplification of GAPDH gene as a reference on a 1.5% agarose gel, had proven the quality of RNA and cDNA products (Figure 3). Pooled samples (2 samples per group) were sequenced based on high throughput sequencing and by using of Illumia Hiseq 4000 platform. Almost 2-30 million paired-end raw read profiles were obtained per sample from the company. All reads passed the preprocessing cutoff thresholds using FastQC software and only the adaptor contaminations were trimmed off by using Trimmomatic software.

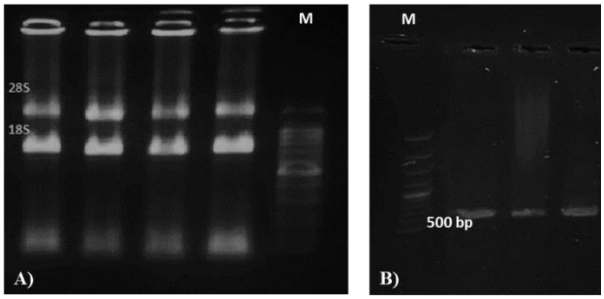

Figure 3. The integrity of RNA and complementary DNA amplification (cDNA)**.** A) The RNA quality of samples based on 28S and 18S ribosomal RNA. B) cDNA quality with amplification of the *GAPDH* gene. Lane M shows 100 bp plus DNA marker.

Differential expression analysis

Read mapping was carried out using STAR aligner, a splice junction aware software. Most of the reads (60.7-79.5%) were mapped to the reference genome. The result of the DE analysis suggested 443 unique genes that were differentially expressed in the infected group, compared to the healthy group. The identification of significant genes was based on the FDR ≤0.05. Around 371 genes were over-expressed with positive Log2–fold change more than 2, and 72 genes were under-expressed with negative Log2 fold change less than 2, in infected samples versus control samples. The detailed information of the discovered genes is presented in Supplementary data file sheet1.

Functional GO terms and pathway analysis

The Gene Ontology enrichment analysis was accomplished for better understanding of the biological processes and involved pathways of identified DEGs in EBL malignancy. There were 112 significant improved GO terms based on FDR ≤ 0.05 (30, 8 and 74 for CC, MF and BP categories, respectively) (see supplementary data file sheet 2). Because of redundancy and overlapping among identified GO term results, they were classified using GO slim method in CateGOrizer online tool to describe the basic terms and relationships between terms within the context of biology (Table 1). A term

Table 1. The classified Gene Ontology (GO) terms of DEGs, using CateGOrizer online tool.

Parent GO ID	GO Definitions	GO terms	Counts	Fractions
GO:0008150	biological-process	BP	74	36.63%
GO:0008152	metabolism	BP	64	14.36%
GO:0009058	biosynthesis	BP	29	11.39%
GO:0006139	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	BP	23	1.49%
GO:0016043	cell organization and biogenesis	BP	3	1.49%
GO:0006259	DNA metabolism	BP	3	0.99%
GO:0006996	organelle organization and biogenesis	BP	2	0.99%
GO:0007049	cell cycle	BP	2	0.50%
GO:0007165	signal transduction	BP	1	0.50%
GO:0007154	cell communication	BP	1	14.36%
GO:0005575	cellular_component	CC	29	41.43%
GO:0005623	cell	CC	16	22.86%
GO:0005622	intracellular	CC	13	18.57%
GO:0005576	extracellular region	CC	5	7.14%
GO:0005634	nucleus	CC	4	5.71%
GO:0005856	cytoskeleton	CC	1	1.43%
GO:0005737	cytoplasm	CC	1	1.43%
GO:0005730	nucleolus	CC	1	1.43%
GO:0003674	molecular_function	MF	8	36.36%
GO:0005488	binding	MF	8	36.36%
GO:0003676	nucleic acid binding	MF	3	13.64%
GO:0003723	RNA binding	MF	2	9.09%
GO:0000166	nucleotide binding	MF	1	4.55%

Only the basic (parent) GO terms are presented. BP, biological process; CC, cellular component; MF, molecular function; Counts are the number of identified GO terms in each class term; Fractions are the percentage of identified GO terms that grouped in each class term.

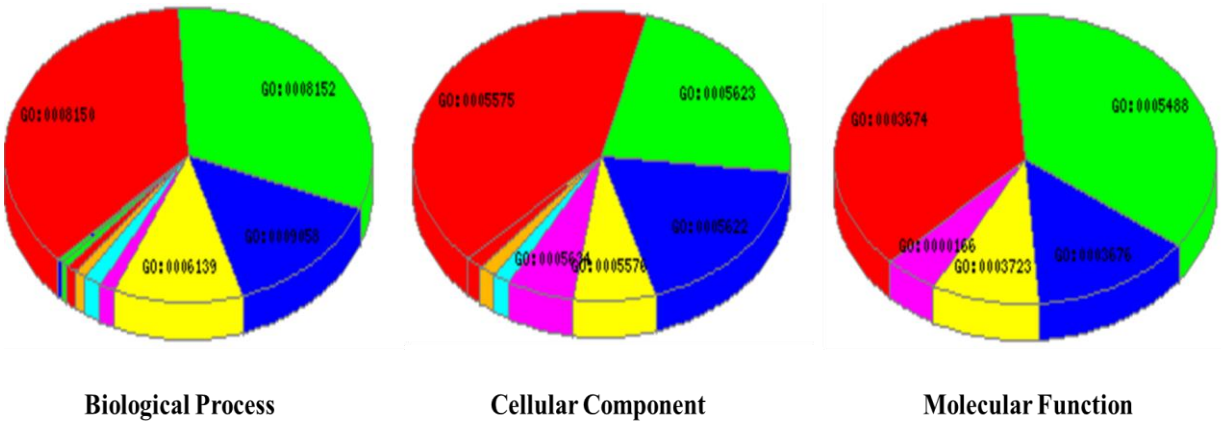

Figure 4. The classified basic Gene Ontology (GO) terms in pie graphs.

may classify in a subset of different terms. The presented percentage in the fraction column of Table 1, express the importance of the identified basic (parent) terms that consisted of child terms. The two top basic terms were metabolism and biosynthesis in BP category, cell and intracellular in CC category, binding and nucleic acid binding in MF category (Figure 4). Our result suggested 20 significant KEGG pathways based on statistically significant *P*-value ≤ 0.01 (Dahiru, 2008) (Table 2). The five top significant KEGG pathways were PI3K-Akt signaling pathway, pathway in cancer, biosynthesis of antibiotics, proteoglycans in cancer, cardiac muscle contraction and B cell receptor (BCR) signaling pathway, in EBL group.

Table 2. The enriched KEGG pathways of DEGs by using DAVID online tool

KEGG ID	Pathway	Counts	%	Pop hits	P-value
bta04151	PI3K-Akt signaling pathway	28	6.378	347	1.33E-05
bta05200	Pathways in cancer	30	6.833	398	2.18E-05
bta01130	Biosynthesis of antibiotics	20	4.555	206	2.74E-05
bta05205	Proteoglycans in cancer	19	4.328	203	7.49E-05
bta04260	Cardiac muscle contraction	11	2.505	80	2.04E-04
bta04662	B cell receptor signaling pathway	10	2.277	70	3.42E-04
bta04916	Melanogenesis	11	2.505	99	0.001
bta04310	Wnt signaling pathway	13	2.961	137	0.001
bta05166	HTLV-I infection	19	4.328	267	0.002
bta04912	GnRH signaling pathway	9	2.050	86	0.005
bta04512	ECM-receptor interaction	9	2.050	87	0.006
bta04020	Calcium signaling pathway	14	3.189	189	0.007
bta05217	Basal cell carcinoma	7	1.594	55	0.007
bta05219	Bladder cancer	6	1.366	40	0.008
bta04915	Estrogen signaling pathway	9	2.050	98	0.01
bta00010	Glycolysis / Gluconeogenesis	7	1.594	63	0.01
bta04510	Focal adhesion	14	3.189	208	0.01
bta04660	T cell receptor signaling pathway	9	2.05	105	0.01
bta05414	Dilated cardiomyopathy	8	1.82	86	0.01
bta04611	Platelet activation	10	2.27	127	0.01

Only the significantly enriched KEGG pathways ($P \leq 0.01$) are presented. Counts are the number of unique DAVID gene symbol corresponding to our input gene list; Pop hits, the number of genes that belong to the specific gene category.

Discussion

The development and progression of leukemogenesis induced by BLV, depend on the level of viral factors such as *Tax* gene expression at the early stage of infection, while some recent studies have been reported that besides of infection, the immune deterioration, growth factor production and activation of cancer driver genes can induce cell transformation and malignancy to form acute phase of infection (Durkin et al., 2016; Gillet et al., 2007). Hence, the study of bovine's transcriptome at the acute phase is helpful for a better understanding of involved genes and pathways in the manifestation of the EBL.

In this study, we produced high throughput transcriptome data using RNA-seq technique from 4 pooled RNA samples to examine gene expression changes in lymph node cells of two EBL samples in the counterpart of two sera-negative samples in response to BLV infection and progression to the lymphocytic stage. The expression of 443 unique DEGs was changed through BLV infection, 371 were up- and 72 were downregulated. Of note, previous studies were applied microarray technique (Brym and Kamiński, 2017; Everts et al., 2005; Klener et al., 2006) for investigation of gene expression changes, while we used RNA-seq technique for the first time. There are only a few DEGs in common between our result and the DEG lists of some microarray studies (e.g *ADORA2B*, *CD19*, *APEX1*, *HIF1A*, *CCT5*, *LMO2*, *HADH*, *DYNLL1*, *C1QBP*, *BCL11A*, *SYK*, *PHGDH*, *WDFY4*, *NMI*, *CXXC5*, *SOX5*, *CYB5R3*, *RHNO1*, *YWHAG*, *LYN*, *Src*, *PEBP1*, *GNL3*, *CXXC5*, *ERH*, *AVEN*, *CMTM7*, *NACA*, *HSPA14*, *ENOPH1*, *LCN2*, *HMGB1*, *CXCL10*, *DYNLL1*, *CXHXorf57*, *ZBTB32*, *TUBB4B*, and *PKM*) and a few DEGs with invert expression (e.g. *HSPA4*, *IL17R*, *CDK4*, *RNASE6*, *SDCBP*, *GAPDH*, *RGS2*, *MKI67*, *TOP2A*, and *CD79A*) (Brym and Kamiński, 2017; Everts et al., 2005; Klener et al., 2006). It is interesting that genes associated with several human malignancies, especially B-cell tumor, were represented among the differentially expressed genes. Some genes were

new and had no recognized role in tumor development.

The upregulated *CXCL10* was significantly associated with tumor cell migration and progression in diffuse large B-cell lymphoma (DLBCL) (Burny et al., 1988) and is a valid biomarker in progress of heart failure (Greenwood et al., 2018). In the EBL samples, the pathway of cardiac muscle contraction was significant, and heart failure might be a sign of clinical symptom of EBL malignancy. The upregulation of *TOP2A* and *MKI67* in prostate cancer (Malhotra et al., 2011) and *CDK4* in B cell lymphoma (Brym and Kamiński, 2017) are resulted to increase the proliferation of malignant cell and they might be good candidates for targeted cancer therapy.

B cells are the main arm of humoral immunity, which is targeted by BLV, thus BCR and three membranes of B cell antigen receptors complex, *CD79A*, *CD19*, and *CD70A*, as most pivotal activators were upregulated in infected BLV cells (Brym and Kamiński, 2017). This strategy of BLV for B cell survival via BCR signaling (Burny et al., 1988) is the homolog of the activation of TCR signaling in HTLV-1 infection (Ghezeldasht et al., 2013) to induce cell cycle promoting proteins, provides growth factors in cell survival to inhibit apoptosis, and activates transforming pathways for malignancy. Activation of the BCR complex leads to the phosphorylation of proto-oncogenes tyrosine kinases family, including *BTK* and SYK in hematopoietic cells (Gauld and Cambier, 2004). High expression of *BTK* and *SYK* genes increase the activation, proliferation, and survival of DLBCL (Gauld and Cambier, 2004; Tolar et al., 2005).

Further, the identified differentially expressed genes were used in the gene enrichment analysis by using the online DAVID database to screen functional patterns associated with the expression differences observed. In general, our result suggested 74 BP, 30 CC and 8 MF. Because of the existence of more similarity and redundancy among obtained Go terms, we categorized GO terms in terms of basic GO classes using CateGorizer online tool based on GO slim method to reveal the main basic 'parent' GO terms and the relationships between 'child' terms (Hu et al., 2008). Finally, whole expressed genes were localized in 7 cellular component term including cell, intracellular or extracellular region, nucleus, cytoskeleton, cytoplasm and nucleus; and were involved in 9 biological processes including metabolism; biosynthesis; nucleobase, nucleoside, nucleotide and nucleic acid metabolism; cell organization and biogenesis; DNA metabolism; organelle organization and biogenesis; cell cycle; signal transduction and cell communication which they mediated four molecular functions including binding; nucleic acid binding; RNA binding and nucleotide-binding. These enrichment results provided further supports to previously published results about the invasion of BLV infected cells (Brym and Kamiński, 2017; Everts et al., 2005; van den Heuvel et al., 2005).

Furthermore, our study suggests the expression changes in genes from 20 distinct KEGG pathways, including PI3K-Akt signaling pathway; pathway in cancer; biosynthesis of antibiotics; proteoglycans in cancer; cardiac muscle contraction; B cell receptor signaling pathway; melanogenesis; Wnt signaling pathway; HTLV-1 infection; GnRH signaling pathway; ECM-receptor interaction; calcium signaling pathway; Basal cell carcinoma; Bladder cancer; estrogen signaling pathway; glycolysis/gluconeogenesis; focal adhesion; T-cell receptor signaling pathway; dilated cardiomyopathy and platelet activation. The dysregulation and activation of mentioned KEGG pathways, leading to the disruption of B-cell survival and growth control. Interestingly, these activated processes were reported previously with HTLV-1, a T-cell-tropic oncoretrovirus, as well as non-virus-associated human B-cell malignancies (Liu et al., 2018; Mozhgani et al., 2018).

The activation of cellular adhesion molecules (CAMs) and focal adhesion process play central roles in transferring and localization of leukemic cells in different tissues that finally affect cancer cell growth and metastasis (Brym and Kamiński, 2017; Burny et al., 1988). More studies are needed to explain the impact of the adhesion process in BLV infiltration to host cells. The glycolysis/gluconeogenesis pathway happens inside the cells to produce adenosine triphosphate (ATP), as a cell energy, to drive many processes in cells. Recent studies have reported the high activation of glycolysis/gluconeogenesis, PI3K-AKT signaling pathway, B cell and T cell receptor signaling pathway in cancer cells, including colorectal cancer, adult T cell leukemia (ATLL), and diffuse large B cell lymphoma (DLBCL) compared to normal cells (Caro et al., 2012; Liu et al., 2018; Mozhgani et al., 2018). Our results indicated that the glycolysis metabolic process was activated in B cells that confers growth and survival advantages in the tumor.

In conclusion, a wide range of effective, up- and downregulated genes involved in EBL pathogenicity and malignancy were introduced by the present study. More identified processes including ECM-

receptor interaction, focal adhesion, BCR signaling, TCR signaling, glycolysis/gluconeogenesis, PI3K-Akt pathway and the dysregulated genes including *CXCL10*, *BTK*, *SYK*, *IL17R* and *CDK4* were associated with the disruption of natural DNA replication and cell proliferation which induce the repression of DNA damage responses and apoptosis in B-cells to make malignancy. Moreover, the over-activation of glycolysis/gluconeogenesis pathway along with immune cells is required to support the metabolic reprogramming of B cells. As regards EBL malignancy is a life-threatening disease for farm animals, more researches on transcriptome level are necessary to accelerate our findings of the EBL diseases.

Acknowledgments

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Conflict of interest

All authors report no conflict of interest.

References

Aida Y., Murakami H., Takagashi M. and Takeshima S.-N. (2013) Mechanisms of pathogenesis induced by bovine leukemia virus as a model for human T-cell leukemia virus. Frontiers in microbiology 4:328.

Andrews S. (2016) FASTQC: A quality control tool for high throughput sequence data. Available from http//www.bioinformatics.babraham.ac.uk/projects/fastqc.

Arainga M., Takeda E. and Aida Y. (2012) Identification of bovine leukemia virus tax function associated with host cell transcription, signaling, stress response and immune response pathway by microarray-based gene expression analysis. BMC genomics 13:121.

Bolger A. M., Lohse M. and Usadel B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120.

Brym P. and Kamiński S. (2017) Microarray analysis of differential gene expression profiles in blood cells of naturally BLV-infected and uninfected Holstein–Friesian cows. Molecular biology reports 44:109-127.

Burny A., Cleuter Y., Kettmann R., Mammerickx M., Marbaix G., Portetelle D., Van den Broeke A., Willems L. and Thomas R. 1988. Bovine leukaemia: facts and hypotheses derived from the study of an infectious cancer. *In* Virus Infections and the Developing Nervous System. Springer. 37-56.

Caro P., Kishan A. U., Norberg E., Stanley I. A., Chapuy B., Ficarro S. B., Polak K., Tondera D., Gounarides J. and Yin H. (2012) Metabolic signatures uncover distinct targets in molecular subsets of diffuse large B cell lymphoma. Cancer cell 22:547-560.

Dahiru T. (2008) P-value, a true test of statistical significance? A cautionary note. Annals of Ibadan postgraduate medicine 6:21-26.

Dequiedt F., Kettmann R., Burny A. and Willems L. (1995) Mutations in the p53 tumor-suppressor gene are frequently associated with bovine leukemia virus-induced leukemogenesis in cattle but not in sheep. Virology 209:676-683.

Dobin A., Davis C. A., Schlesinger F., Drenkow J., Zaleski C., Jha S., Batut P., Chaisson M. and Gingeras T. R. (2013) STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15-21.

Durkin K., Rosewick N., Artesi M., Hahaut V., Griebel P., Arsic N., Burny A., Georges M. and Van den Broeke A. (2016) Characterization of novel Bovine Leukemia Virus (BLV) antisense transcripts by deep sequencing reveals constitutive expression in tumors and transcriptional interaction with viral microRNAs. Retrovirology 13:33.

Everts R. E., Band M. R., Liu Z. L., Kumar C. G., Liu L., Loor J. J., Oliveira R. and Lewin H. A. (2005) A 7872 cDNA microarray and its use in bovine functional genomics. Veterinary immunology and immunopathology 105:235-245.

Gauld S. B. and Cambier J. C. (2004) Src-family kinases in B-cell development and signaling. Oncogene 23:8001.

Ghezeldasht S. A., Shirdel A., Assarehzadegan M. A., Hassannia T., Rahimi H., Miri R. and Rezaee S. R. (2013) Human T lymphotropic virus type I (HTLV-I) oncogenesis: molecular aspects of virus and host interactions in pathogenesis of adult T cell leukemia/lymphoma (ATL). Iranian journal of basic medical sciences 16:179.

Gillet N., Florins A., Boxus M., Burteau C., Nigro A., Vandermeers F., Balon H., Bouzar A.-B., Defoiche J. and Burny A. (2007) Mechanisms of

leukemogenesis induced by bovine leukemia virus: prospects for novel anti-retroviral therapies in human. Retrovirology 4:18.

Greenwood A. D., Ishida Y., O'Brien S. P., Roca A. L. and Eiden M. V. (2018) Transmission, evolution, and endogenization: lessons learned from recent retroviral invasions. Microbiology and molecular biology reviews 82:e00044-00017.

Hu Z.-L., Bao J. and Reecy J. M. (2008) CateGOrizer: a web-based program to batch analyze gene on-tology classification categories. Online journal of bioinformatics 9:108-112.

Huang D. W., Sherman B. T. and Lempicki R. A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols 4:44.

Juliarena M. A., Barrios C. N., Lützelschwab C. M., Esteban E. N. and Gutiérrez S. E. (2017) Bovine leukemia virus: current perspectives. Virus adaptation and treatment 9:13-26.

Klener P., Szynal M., Cleuter Y., Merimi M., Duvillier H., Lallemand F., Bagnis C., Griebel P., Sotiriou C. and Burny A. (2006) Insights into gene expression changes impacting B-cell transformation: cross-species microarray analysis of bovine leukemia virus tax-responsive genes in ovine B cells. Journal of virology 80:1922-1938.

Konnai S., Usui T., Ikeda M., Kohara J., Hirata T.-i., Okada K., Ohashi K. and Onuma M. (2006) Tumor necrosis factor-alpha genetic polymorphism may contribute to progression of bovine leukemia virus-infection. Microbes and infection 8:2163-2171.

Kouznetsova V., Chen S. and Tsigelny I. (2019) HTLV-1 can be involved in acceleration of different nonhematological cancers. Journal of cancer research and therapeutics 7:1-8.

Li H., Handsaker B., Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G. and Durbin R. (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25:2078-2079.

Liao Y., Smyth G. K. and Shi W. (2013) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30:923-930.

Liu Z., Meng J., Li X., Zhu F., Liu T., Wu G. and Zhang L. (2018) Identification of hub genes and key pathways associated with two subtypes of diffuse large B-cell lymphoma based on gene expression profiling via integrated bioinformatics. BioMed research international 2018.

Love M. I., Huber W. and Anders S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome biology 15:550.

Malhotra S., Lapointe J., Salari K., Higgins J. P., Ferrari M., Montgomery K., van de Rijn M., Brooks J. D. and Pollack J. R. (2011) A tri-marker proliferation index predicts biochemical recurrence after surgery for prostate cancer. PLoS One 6:e20293.

Moratorio G., Fischer S., Bianchi S., Tomé L., Rama G., Obal G., Carrión F., Pritsch O. and Cristina J. (2013) A detailed molecular analysis of complete bovine leukemia virus genomes isolated from B-cell lymphosarcomas. Veterinary research 44:19.

Mousavi S., Haghparast A., Mohammadi G. and Tabatabaeizadeh S.-E. 2014. Prevalence of bovine leukemia virus (BLV) infection in the northeast of Iran. *In* Veterinary research forum: an international quarterly journal. Vol. 5. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. 135.

Mozhgani S. H., Zarei-Ghobadi M., Teymoori-Rad M., Mokhtari-Azad T., Mirzaie M., Sheikhi M., Jazayeri S. M., Shahbahrami R., Ghourchian H. and Jafari M. (2018) Human T-lymphotropic virus 1 (HTLV-1) pathogenesis: A systems virology study. Journal of cellular biochemistry 119:3968-3979.

Polat M., Takeshima S.-n. and Aida Y. (2017) Epidemiology and genetic diversity of bovine leukemia virus. Virology journal 14:209.

Rola-Łuszczak M., Finnegan C., Olech M., Choudhury B. and Kuźmak J. (2013) Development of an improved real time PCR for the detection of bovine leukaemia provirus nucleic acid and its use in the clarification of inconclusive serological test results. Journal of virological methods 189:258-264.

Tolar P., Sohn H. W. and Pierce S. K. (2005) The initiation of antigen-induced B cell antigen receptor signaling viewed in living cells by fluorescence resonance energy transfer. Nature immunology 6:1168.

Van den Heuvel M. J., Jefferson B. J. and Jacobs R. M. (2005) Purified bovine plasma blocking factor decreases Bovine leukemia virus p24 expression while increasing protein synthesis and transcriptional activity of peripheral blood

mononuclear cells in short-term culture. Canadian journal of veterinary research 69:186.