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Oncogenic miR-146a-*PBX2* axis, a novel leukemic target in xenograft acute lymphoblastic leukemia model

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Abstract
Acute lymphoblastic leukemia (ALL) is one of the major hematological malignancies both in adults and children. Bone marrow biopsy is the main requirement for its accurate diagnostic and prognostic purpose till now that is a very painful process especially for children. The reluctance to go for bone marrow biopsy leads to delayed diagnosis treatment of ALL. MicroRNAs are emerging as diagnostic and therapeutic targets for cancer including ALL using less painful methods. The current study was conducted to evaluate the potential of miR-146a-*PBX2* axis as a leukemic target of ALL using ALL-induced rabbit model. A total of 8 rabbits were divided into two groups: ALL-induced (diseased) and normal (control). The whole blood was collected from both groups and plasma isolation was performed. The levels of miR-146a and *PBX2* gene in plasma of diseased and control samples were quantified by the real-time PCR (RT-qPCR). The normalized fold expression was calculated using miR-16 and the reference gene *GAPDH*. The results showed that there was a significant increase in miR-146a expression in diseased (ALL-induced) samples as compared to the control. Moreover, a significant upregulation of *PBX2* gene was found in the diseased model as compared to that in the control. The results implied that miR-146a-*PBX2* axis may be utilized as a potential target for ALL.

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Introduction

Acute lymphoblastic leukemia (ALL) is one of the malignant blood disorders which originates due to the uncontrolled proliferation of the hematopoietic stem cells. According to the American Cancer Society, about 6,540 new cases of ALL (3,660 in males and 2,880 in females) and 1,390 deaths (700 in males and 690 in females) have been reported in the US (Shinde and Srivaramangai, 2023). Moreover, about 59,610 people are expected to be diagnosed with leukemia in 2023 (Rudolph and Antoinette, 2023). ALL has been reported to metastasise into CNS, lymph nodes, spleen, liver and kidney elevating symptoms of body pains, fever and anaemia (Portell et al., 2013). Advancements in treatment strategies have significantly improved the lives of ALL patients, but there is still the disease relapse due to genetic

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heterogeneity of the disease (Bellavia et al., 2018). Chromosomal translocation, environmental factors, genetic and epigenetic alterations are the major contributors of ALL. Currently, the dysregulated patterns of microRNAs have been elucidated as one of the major causes of ALL (Xiao et al., 2017). Numerous studies have confirmed altered expression of microRNAs in several cancers which can be considered as important non-invasive tools for disease confirmation. Although bone marrow biopsy is the major diagnostic method for ALL, still it is lethargic and time-consuming for patients and medical professionals (Chiaretti et al., 2010). Studies have reported that microRNAs can act as potential diagnostic, prognostic and predictive biomarkers. They are abundant and highly stable in blood circulation and can escape RNAases (Xiong et al., 2014).

The oncogenic potential of miR-146a is reported as it is highly expressed in hematopoietic stem cell regulating immune related functions and oncogenesis (Tavakoli et al., 2016). It regulates proliferation and differentiation of T helper cells, megakaryocytes, platelets and myeloid cells. Upregulation of miR-146a has been observed in stem cell lines of the monocytic and lymphocytic lineages obtained from bone marrow transplant (Starczynowski et al., 2011; Peng-Fei Zhai et al., 2014). Several studies have reported the dysregulated miR-146a in ALL as it has a substantial role in T-cell development by influencing NB4 cell proliferation and death (Zhang et al., 2009; Hua et al., 2011; Duyu et al., 2014).

PBX2 is known to modulate cell proliferation, differentiation and apoptosis in cancer cells (Jianjiao Lin et al., 2021). Its over-expression is correlated with poor prognosis in various cancers. The increased expression level of *PBX2* gene is correlated with poor prognosis in lung and gastric cancer (Jianjiao Lin et al., 2021). Overly expressed *PBX2* gene activates *BCL-2*, an anti-apoptotic gene, suggesting *PBX2* may be involved in cancer initiation and progression (Blackwell et al., 2009; Qiu et al., 2010, 2012). According to the *in silico* analysis, *PBX2* gene upregulates *c-Myc*, a pro-oncogene which is a potent regulator of hematopoiesis. The role of *PBX2* gene is yet to be discovered, although this gene has been reported to cause leukemogenesis, since it is responsible for chromosomal translocation in acute pre-B-cell leukaemia (Dasse et al., 2012). However, the exact role of *PBX2* gene in ALL is yet to be discovered (Shahid et al., 2021). There are fewer studies unfolding the role of *PBX2* gene, therefore, studies on expression analysis of miR-146a-*PBX2* axis is of utmost importance to get insights into its role in leukemogenesis. In a previous study, *PBX2* gene is predicted to be a novel target of miR-146a in ALL via *in silico* approach (Shahid et al., 2021). The present research was designed to experimentally validate the oncogenic role of miR-146a along with its potent target, *PBX2* gene, in the plasma samples of ALL-induced xenograft rabbit model. The miR-146a-*PBX2* expression levels were specifically detected using RT-qPCR in a xenograft ALL-induced rabbit model.

Materials and Methods

This study was conducted at the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan. This research was conducted according to the principles and guidelines of the Declaration of Helsinki ensuring minimal pain and discomfort to the study subjects.

Animal model development

To develop an animal model of ALL, a total of 8 immuno-compromised rabbits of 1 kg body weight were maintained under standard conditions. All rabbits were kept at the animal house, the University of Lahore. After one week of normalization, rabbits were categorized into two groups, *i.e*., group 1: diseased (n = 4), and group 2: Control (n = 4). All rabbits were given chow, vegetables and water in order to provide appropriate diet. ALL rabbit model was prepared using plasma of ALL patients by the xenograft modelling method. Four rabbits were induced with ALL patient plasma and 4 were kept as control. All animals were monitored daily to check healthy eating and drinking habits. Body weight, body temperature and breathing rate were recorded on every third day to ensure the progressive development of leukemia (Meyer and Debatin, 2011).

Sample collection

Blood samples (each 10 mL) from each rabbit were collected in EDTA tubes for haematological analysis. Plasma was separated after double centrifugation of the whole blood and stored in 1.5 mL nuclease free tubes at -80 ℃ for microRNA expression studies.

Hematological investigations

The EDTA containing blood samples were analysed for hematological parameters including complete blood count (CBC) and peripheral blood smear following protocols provided with commercial kits.

RNA isolation

Total RNA was isolated from the rabbit plasma using TRIzol reagent (Thermo Fisher Scientific -US).

RNA integrity of samples was checked at A260/280 nm using the Thermo Scientific™ NanoDrop™ One Spectrophotometer. Isolated RNA was aliquoted and stored at -80 ℃ until further use.

Reverse transcription

Total RNA was reverse transcribed into cDNA using the RevertAid First Strand CDNA Synthesis Kit (Cat. # K1622) from Thermo Fisher Scientific according to the manufacturer's instructions. The reaction was carried out at 25 ℃ for 5 minutes, at 42 ℃ for 60 minutes and at 65 ℃ for 20 minutes using Thermal Cycler (v2.09) from Applied Biosystems. The prepared cDNA was immediately stored at -20 ℃ for subsequent real time PCR.

Primer designing

The sequences of target miR-146a and reference miR-16 were retrieved from miRbase (www.mirbase.org). The target *PBX2* gene along with its reference *GAPDH* gene sequences were taken from the NCBI (www.ncbi.nlm.nih.gov). The primers were designed using miRprimer (v2.0) and Primer3web (v4.1.0) tools, and then synthesized commercially. The accession number of primers are: miR-146a (MIMAT0000449), miR-16 (MIMAT0000069), *PBX2* (NC_000006) and *GAPDH* (NC_000012).

Quantitative real time PCR

Following the reverse transcription, quantitative real time PCR (qPCR) was performed to analyze miR-146a and *PBX2* gene expression level using SYBR green PCR master mix from Thermo Fisher Scientific (Cat. # 4309155). The reaction was carried out using the StepOne Plus Real Time PCR system (Thermo Fisher Scientific-US). All the reactions were performed in duplicate under the following cycling conditions: 95 ℃ for 5 mins, 95 ℃ for 15 secs (40 cycles), 62 ℃ (Tm) for 1 min. The qPCR master mix contained: 1 µL of cDNA, 10 µL of 2X SYBR green PCR mix and 0.5 µL of each forward and reverse miR-146a and *PBX2* gene specific primers. The final volume was made up to 20 µL using nuclease-free water. The Ct (cycle threshold) values of diseased samples were compared with that of the control to achieve relative quantification. The Ct of miR-146a was normalized to miR-16a, and that of *PBX2* was GAPDH that served as a reference gene. The normalized fold expression level of miR-146a and *PBX2* gene was calculated with a formula of $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). The RT-qPCR was performed in duplicate, including no-template controls to check for any contamination.

Networking and target prediction

The potential targets of miR-146a and *PBX2* gene were predicted using data information from miRTarBase (https://mirtarbase.cuhk.edu.cn/). The associated gene ontology and gene-gene networks were constructed using the STRING database (version 12.0) (http://string-db.org/) and Cytoscape software (version 3.10) to validate interacting target genes for *PBX2*.

Statistical analysis

The results were presented as mean ± standard deviation. The SPSS statistical software package version 10.0 (SPSS Inc., Chicago Il, USA) was used to analyse significant difference in haematological parameters between ALL-induced and control samples using Mann–Whitney U test. Statistical analyses for qRT-PCR were performed using Graphpad prism 9. The *P*-values < 0.05 were considered statistically significant.

Results

Physical examination

Physical assessment was performed by evaluating body temperature, body weight and breathing rate of ALL-induced rabbits (n = 4) and compared it with the control rabbits (n = 4). The body temperature of induced ALL model was 102.5 ºF ± 0.33 as compared to the control with 99.95 ºF ± 0.10. There was a significant difference in body temperature, weight and breathing rate of control (R1) and ALL-induced (R2) group (**Table 1**).

Table 1. Physical parameters of the animal model

Parameter	Control (R1)	ALL-induced (R2)	*P*-value
Body temperature (Ŧ)	99.95 ± 0.10	102.5 ± 0.33	< 0.0001
Body weight (kg)	0.74 ± 0.01	0.67 ± 0.01	0.0006
Breathing rate/min	96.00 ± 0.81	110.8 ± 0.95	< 0.0001

*P < 0.05 showed statistically significant differences

Hematological investigations

The results of hematological examination showed a significant reduction in haemoglobin (Hb), RBCs and platelets ($P < 0.05$) as shown in **Table 2**. However, a significant increase in WBCs was observed in ALL-induced rabbit model (R2) compared to that in the control (R1) (**Table 2**). The results illustrated mild anemia and leukocytosis that is the most common abnormal hematological parameter associated with ALL patients. The decreased platelets showed the presence of thrombocytopenia due to leukemic cells infiltrating the bone marrow tissue of the ALL model.

Table 2. Hematological investigations of control vs ALL-induced group

Hematological parameters	Control (R1)	ALL-induced (R2)	*P*-value
Hb (g/dL)	12.48 ± 0.09	8.63 ± 0.25	< 0.0001
RBC ($x10^6$/µL)	4.88 ± 0.18	3.40 ± 0.35	0.0003
HCT (%)	34.25 ± 0.50	27.00 ± 0.82	< 0.0001
MCV (fL)	67.50 ± 1.29	74.13 ± 0.83	0.0001
MCH (pg)	19.68 ± 0.29	21.75 ± 0.46	0.0003
MCHC (g/dL)	28.13 ± 0.82	29.30 ± 0.61	0.0604
PLT ($x10^3$/µL)	239.10 ± 6.25	122.50 ± 1.92	< 0.0001
WBC ($x10^3$/µL)	3.57 ± 1.21	6.65 ± 0.90	0.0286
Neutrophils (%)	65.50 ± 1.73	75.25 ± 3.59	0.0027
Lymphocytes (%)	28.08 ± 0.43	21.25 ± 0.86	< 0.0001
Monocytes (%)	2.12 ± 0.09	4.15 ± 0.31	< 0.0001
Eosinophil (%)	2.15 ± 0.13	1.83 ± 0.17	0.0229

*$P < 0.05$ showed statistically significant differences.

Peripheral blood test

The blood test showed the presence of more than 5% blasts in the peripheral blood smear of diseased group, *i.e.*, ALL-induced rabbit model as shown in **Figure 1a**. However, there were no blast cells in the peripheral blood smear of the control group (**Figure 1b**). The appearance of lymphoblasts in the blood of the diseased group confirmed the presence of ALL.

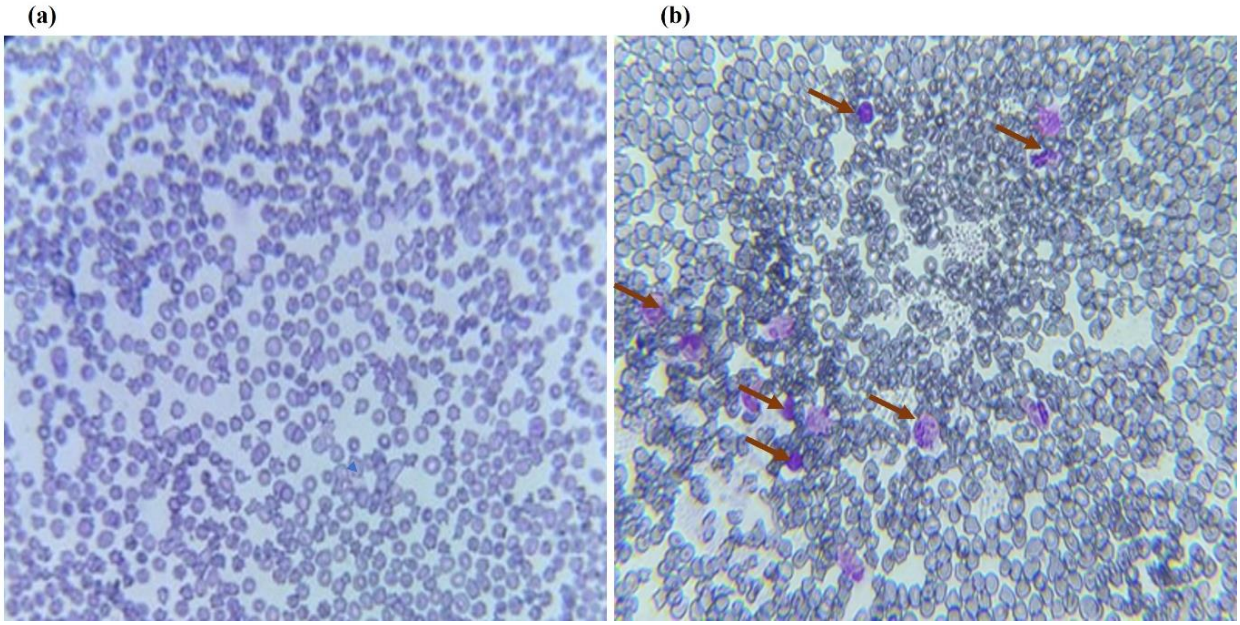

Figure 1. Peripheral blood smear test: (a) Peripheral blood picture of the control group showed normal morphology of RBCs, WBCs, platelets and no lymphoblasts observed under 40X light microscope. (b) ALL-induced group showed the presence of lymphoblasts (> 5%) in the peripheral blood picture observed under 40X light microscope represented by red arrow signs.

Histopathological examination

For the validation of the ALL model, bone marrow biopsy of the diseased and control groups was done. The histopathological examination of the bone marrow biopsy showed more than 5% lymphoblasts (**Figure 2a**). On the contrary, not a single blast was found in the normal bone marrow samples from the control group (**Figure 2b**). These results validated the development of ALL in xenograft induced rabbit model.

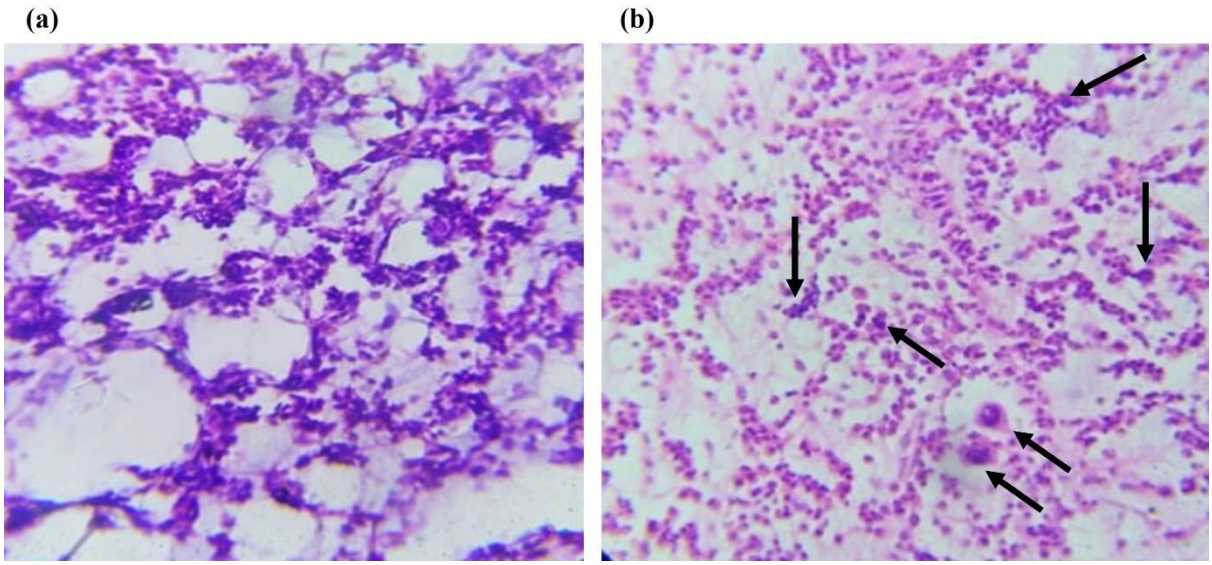

Figure 2. Histopathological examination of bone marrow biopsy: (a) Bone marrow of the control group showed normal morphology with more adipocytes as compared to those in the diseased group. There were no blast cells seen; (b) Bone marrow of the ALL-induced group showed the abnormal morphology with the presence of lymphoblasts as depicted by black arrow signs. The bone marrow biopsy sample was observed under 40X light microscope.

Expression analysis of miR-146a and *PBX2* gene

To investigate the relative expression level of miR-146a and *PBX2* gene in the ALL-induced rabbit model, we evaluated its normalized fold expression according to the method introduced by Livak and Schmittgen in 2001. There was a significant upregulation of miR-146a and *PBX2* gene by 1-fold in the plasma of the ALL-induced rabbit model compared to that in the control group. The mean fold expression of miR-146a was 102.0, while that of *PBX2* gene was 9.19 compared to that of the control group (**Figure 3a-b**).

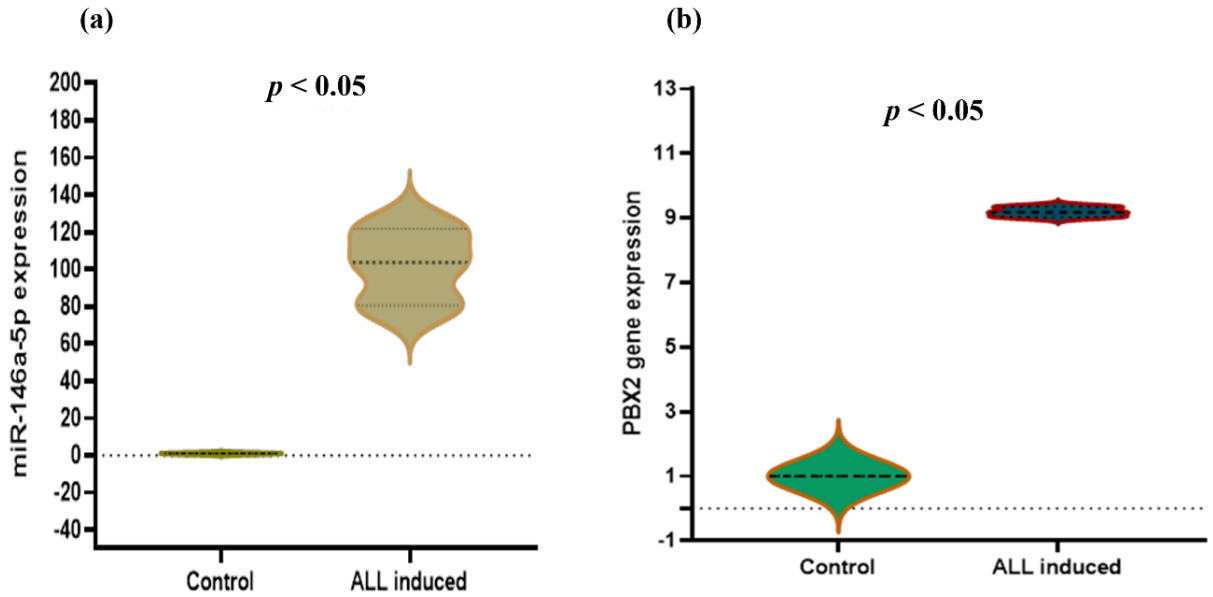

Figure 3. Expression analysis of miR-146a and *PBX2* gene using the real time quantitative PCR. (a) The violon graph showed the normalized fold expression of miR-146a in the control and ALL-induced (diseased) group. The graph illustrates a significant upregulation with a mean 102-fold expression in the diseased group as compared to that in the control; and (b) the violon graph of *PBX2* gene with 9.19-fold expression in the plasma of the ALL-induced rabbit model (diseased) compared to the control. The Y-axis represents normalized fold expression level of miR-146a and *PBX2* gene.

Networking and target prediction

Target information for the miR-146a collected from the miRTarBase (https://mirtarbase.cuhk.edu.cn/) and STRING database (version 12.0) (http://string-db.org/) showed *BCL2A1, BRCA1, BRCA2, BRMS1, CCNA2, CD40LG, CDKN1A, CDKN3, CFH, CXCR4, EGFR, ERBB4, FADD, FAF1, FAS, HOXD10, IL6, IL8, IRAK1, IRAK2, IRF7, KIF22, MTA2, NFIX, NFKB1, PA2G4, PBX2, RAD54L, ROCK1, SMAD4, SNAP25, STAT1, TLR2, TLR4,* and *TRAF6* as the associated and possible target genes (**Figure 4a**). These genes were the top 15 targets with the probability > 0 and the gene symbols were converted according to the official HUGO Gene Nomenclature Committee guidelines (https://www.genenames.org/). Repeated genes were eliminated and the target list was uploaded to the Cytoscape software (version 3.10.). The built-in software then constructed the associated gene ontology and gene-target-gene network to obtain pivotal targets for miR-146a according to the network parameters. The protein interaction network showed that *AGPAT1, PKNOX1,* and *SLC38A2* are co-expressed with *PBX2* and *PBX3*. Moreover, *PKNOX2, MEIS2* and *MEIS1* are important paralogs of *PBX2*. The *PBX2* gene is important for the activation of *HOX11* contributing to the progression of T-cell ALL (**Figure 4b**). The visual interaction between miR-146a and *PBX2* gene confirms the upregulated interaction with *PBX2* oncogene which plays an important role in the progression of ALL. More studies are required for exploring the role of *PBX2* in differential classification of leukemia.

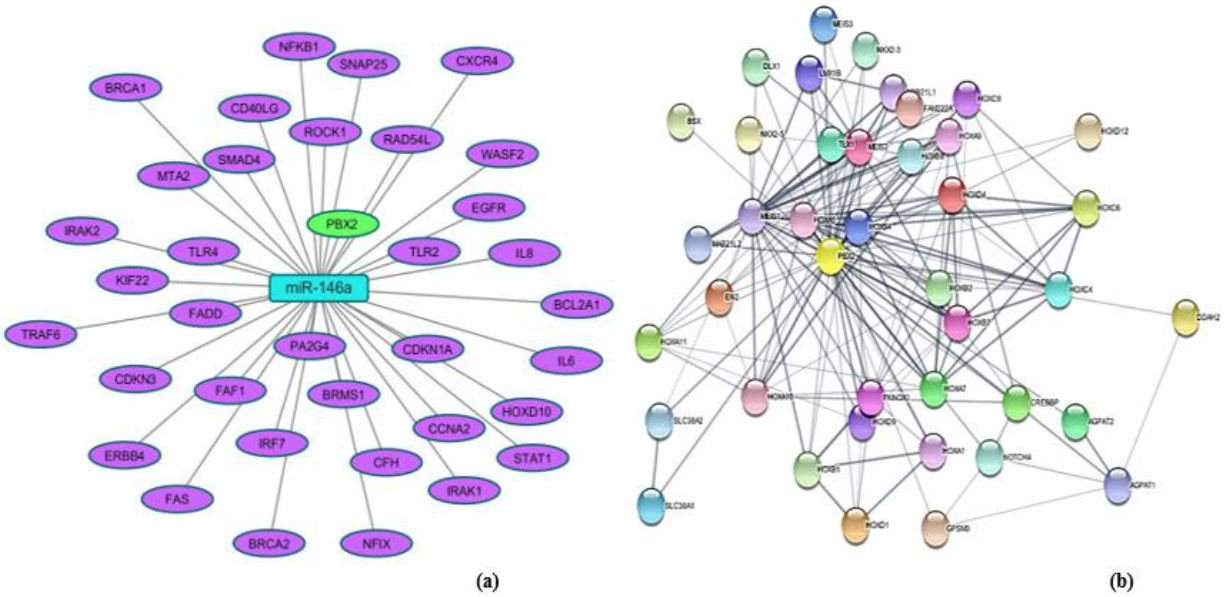

Figure 4. (a) Interaction between miR-146a and *PBX2* gene, highlighting the upregulated interaction with *PBX2* oncogene, which plays an important role in the progression of ALL; the blue coloured box shows the miR-146a, the purple-coloured ovals show the target genes of miR-146a and the green coloured oval shows the *PBX2* gene; (b) String pathway analysis of proteins regulated by *PBX2* gene. Each circle represents all the proteins produced by *PBX2* gene (yellow circle). The protein interaction network showed that AGPAT1, PKNOX1, and SLC38A2 are co-expressed with PBX2 and PBX3. Moreover, PKNOX2, MEIS2 and MEIS1 are important paralogs of PBX2. *PBX2* gene is known to activate *HOX11* contributing to the progression of T-cell ALL.

Discussion

Recent trends in microRNAs research have been increasing rapidly over the past few years due to their ability to express aberrantly in different cellular conditions. Moreover, they are able to differentiate and characterize unique cell types making them valuable diagnostic markers for several diseases. Our goal was to evaluate the expression level of miR-146a since this microRNA has been reported to be dysregulated in haematological malignancies and other cancers (Swellam and El-Khazragy, 2016). Not much work has been done on *PBX2,* but hypothetically it is a direct target of miR-146a involved in leukemia progression (Brake et al., 2002; Shahid et al., 2021). We hypothesized that miR-146a and *PBX2* gene axis may act as potential target for ALL. We evaluated the oncogenic potential of miR-146a and *PBX2* gene by quantifying the expression level in the plasma of the xenograft ALL-induced rabbit model using real time PCR. We observed significant upregulation in both miR-146a and *PBX2* gene. The results were unexpectedly surprising as both miRNA and the gene were significantly upregulated in the plasma samples of the ALL-induced xenograft rabbit model compared to healthy control demonstrating their oncogenic role in the development of leukemia.

The results of miR-146a have shown to be consistent with the previous findings. Previous studies have shown miR-146a to be dysregulated in the plasma of ALL patients. A significant upregulation of miR-146a has been in T-ALL patients compared to the control. Only one study reported downregulation of miR-146a in the blood and bone marrow samples of T-ALL patients, whereas others reported its upregulation (Feng et al., 2016). In another study, miR-146a showed upregulation in monocytic and lymphocytic stem cell lines extracted from the bone marrow samples of ALL patients (Starczynowski et al., 2011). Microarray results confirmed upregulated miR-146a after validation with qPCR in the bone marrow samples of ALL patients, but no upregulation was found in the blood samples (Duyu et al., 2014). However, only one study showed no significant difference in the miR-146a-5p expression level between the blood samples of ALL patient compared to the control (Tavakoli et al., 2016).

The miR-146a has been reported to target several genes which leads to pathogenesis of ALL. *PBX2* is one of the potent novel targets of miR-146a which is known to hypothetically upregulate according to the MetaCore software analysis. *PBX2* gene regulates the expression of additional gene which is involved in cell proliferation, cell growth and development as well as in cell cycle. *PBX2* gene positively regulates *BCL-2*, anti-apoptotic gene, through a series of signalling cascades arresting cellular apoptosis, thereby causing leukemic cells to divide and multiply aggressively. We can speculate that *PBX2* might be involved in the anti-apoptotic activity of leukemic cells, since *PBX2* gene upregulates *BCL-2*. It could be possible that the binding of *PBX2* gene indirectly expresses *BCL-2* itself. *PBX2* gene also binds with *c-Myc* upregulating it through the Notch signalling pathway leading to a rapid progression of ALL (Shahid et al., 2021). It could be possible that the combined activation of miR-146a and *PBX2* gene might be responsible for the progression of ALL.

The presence of the earlier-mentioned upregulated genes upon microRNA overexpression could be an underlying mechanism supressing tumor suppressor genes. Since *PBX2* gene is a transcription factor, so we speculate that its overexpression might downregulate additional genes in the system responsible for ALL progression.

Despite the fact that the PBX proteins were first discovered as transcriptional activators, some data suggest that they also contain a repressive domain upstream of the homeodomain that appears to have independent function from DNA binding. As a result, the functional role of this family of proteins is complicated and needs more research. In addition, *PBX2* gene is often found to function in complex form with other genes such as *MEIS1*. To elucidate its role as a diagnostic marker, it will be beneficial to investigate the panel of genes associated with *PBX2.*

To further discover the interactive proteins for *PBX2* gene, a Cytoscape with STRING database plugin was run. We found additional genes having functional interactions with *PBX2* gene. *PBX2* activates *HOX11* which is known to contribute to the progression of T-cell acute lymphoblastic leukaemia (Brake et al., 2002). Moreover, *AGPAT1, PKNOX1,* and *SLC38A2* were found to be co-expressed with *PBX2*. These are validated targets retrieved from different experimental and text mining databases. However, pathway enrichment analysis of *PBX2* gene is yet to be discovered.

The miR-146a plays a crucial role in haematopoiesis. It plays a role as both, tumor suppressors and oncomiRs with aberrant expression in any of these. This will lead to abnormal haematopoiesis causing several hematopoietic malignancies. Understanding microRNAs mechanisms in haematopoiesis will allow us to elucidate their roles in pathogenesis of ALL. However, the root cause of molecular mechanisms leading to initiation and progression of ALL still needs to be unravelled (Vasilatou et al., 2010). Abnormal effects have been shown on haematopoiesis in miR-146a gene knockout mice model suggesting it to be a critical modulator of hematopoietic stem cell related functions (such as inflammation, cell differentiation, proliferation). It has also shown its role in the development and regulation of mature myeloid cells during innate immune response (Swellam and El-Khazragy, 2016). It is upregulated during inflammatory response via *NFKB* in these cells which then inhibit *NFKB* via downregulating *TRAF6* to suppress inflammation causing simultaneously downregulation of miR-146a suggesting it a tumor suppressor gene. *TRAF6* regulates *MAPK* and *PI3K* pathways which are involved in cell proliferation (Maude and Tasian, 2012). To our knowledge this is the first report presenting miR-146a-*PBX2* gene axis as a potential leukemic target in the xenograft rabbit model, which could give insights into determining the root cause of the ALL disease.

Conclusion

The results indicated that there was a significant increase in miR-146a expression in the diseased (ALL-induced) samples as compared to the control. Moreover, a significant upregulation of *PBX2* gene was found in the diseased model as compared to that in the control. The significant upregulation of miR-146a and its target gene *PBX2* in the plasma of the ALL rabbit model represented their oncogenic role in the development of ALL. Therefore, oncogenic miR-146a-*PBX2* axis may be utilized as a potential target

for ALL. However, further studies are required to validate the use of miR-146a-*PBX2* axis as an oncogenic target in case of ALL. The regulatory role of *PBX2* gene in leukemogenesis is yet to be explained. Future studies may be conducted to reveal regulatory pathways of target genes so as to establish more effective treatment strategies for ALL.

Author(s), Editor(s) and Publisher's declarations

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Conflict of interest
The authors declare no conflict of interest.

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Declared none.

Contribution of authors
Conceptualization and designing the study: SS. Research supervisor: SS. Conduction of the experiment: SS, MJ. Analytical work: SS, MJ. Data collection, visualization and interpretation: SS, MJ, WS, RA, HN, SM. Graphical representation/visualization: SS, MJ, WS, AL, RA, Preparation of initial draft: SS, MJ. Critical revision and approval of the final version: SS, MJ, WS, RA, AL, HN, SM.

Ethical approval
Ethical approval to work on animals was obtained from the Departmental Bioethics, Biosafety and Biosecurity Committee (Ref. # IMBB/BBBC/22/785), IMBB, The University of Lahore, Lahore, Pakistan

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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Declaration of Generative AI and AI-assisted technologies in the writing process
It is declared that we the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

References

Bellavia, D., Palermo, R., Felli, M.P., Screpanti, I., Checquolo, S. (2018). Notch signaling as a therapeutic target for acute lymphoblastic leukemia. *Expert Opinion on Therapeutic Targets* 22(4): https://doi.org/10.1080/14728222.2018.1451840

Brake, R., Kees, U., Watt, P.M. (2002). A complex containing PBX2 contributes to activation of the proto-oncogene *HOX11*. *Biochemical and Biophysical Research Communications* 294(1):23–34.

Chiaretti, S., Messina, M., Tavolaro, S. *et al.* (2010). Gene expression profiling identifies a subset of adult T-cell acute lymphoblastic leukemia with myeloid-like gene features and over-expression of miR-223. *Haematologica* 95(7):1114-1121. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2895035/

Dasse, E., Volpe, G., Walton, D., Wilson, N., Del Pozzo, W., O'Neill, L.P. et al. (2012). Distinct regulation of c-myb gene expression by HoxA9, Meis1 and Pbx proteins in normal hematopoietic progenitors and transformed myeloid cells. *Blood Cancer Journal* 2(6):e76. https://doi.org/10.1038/bcj.2012.20

Duyu, M., Durmaz, B., Gunduz, C., Vergin, C., Karapinar, D. (2014). Prospective evaluation of whole genome microRNA expression profiling in childhood acute lymphoblastic leukemia. *BioMed Research International* 967585. https://doi.org/10.1155/2014/967585

Feng, X., Xue, J., Xie, K. (2016). miR-146 promotes apoptosis of Jurcat cell in children with acute lymphoblastic leukemia by targeting STAT1 expression. *International Journal of Clinical and Experimental Medicine* 9(11):21280–21287.

Hua, Z., Chun, W., Fang-Yuan, C. (2011). MicroRNA-146a and hemopoietic disorders. *International Journal of Hematology* 94(3):224–229. https://doi.org/10.1007/S12185-011-0923-7

Lin, J., Zhu, H., Hong, L., Tang, W., Wang, J., Hu, H. *et al.* (2021). Coexpression of HOXA6 and PBX2 promotes metastasis in gastric cancer. *Aging* 13(5):6606–6624. https://doi.org/10.18632/aging.202426

Livak, K.J., Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25(4):402–408.

Maude, S. L., Tasian, S.K., Vincet, T., Hall, J.W., Sheen, C., Roberts, K.G. et al. (2012). Targeting JAK1/2 and mTOR in murine xenograft models of Ph-like acute lymphoblastic leukemia. *Blood* 120(17):3510–3518. DOI: 10.1182/blood-2012-03-415448

Meyer, L.H., Debatin, K.M. (2011). Diversity of human leukemia xenograft mouse models: Implications for disease biology. *Cancer Research* 71(23):7141–7144. https://doi.org/10.1158/0008-5472.CAN-11-1732

Portell, C., Wenzell, C. (2013). Clinical and pharmacologic aspects of blinatumomab in the treatment of B-cell acute lymphoblastic leukemia. *Clinical Pharmacology: Advances and Applications* 5(Suppl 1):5–11. https://doi.org/10.2147/CPAA.S42689

Qiu, Y., Morii, E., Tomita, Y., Zhang, B., Matsumura, A., Kitaichi, M., Okumura, M., Aozasa, K. (2009). Prognostic significance of pre-B cell leukemia transcription factor 2 (PBX2) expression in non-small cell lung carcinoma. *Cancer Science* 100(7):1198–1209. https://doi.org/10.1111/j.1349-7006.2009.01156.

Qiu, Y., Song, B., Zhao, G., Deng, B., Makino, T., Tomita, Y. et al. (2010). Expression level of Pre B cell leukemia homeobox 2 correlates with poor prognosis of gastric adenocarcinoma and esophageal squamous cell carcinoma. *International Journal of Oncology* 36(3):651-663. https://doi.org/10.3892/ijo_00000541

Qiu, Y., Wang, Z.L., Jin, S.Q., Pu, Y.F., Toyosawa, S., Aozasa, K. et al. (2012). Expression level of pre-B-cell leukemia transcription factor 2 (PBX2) as a prognostic marker for gingival squamous cell carcinoma. *Journal of Zhejiang University. Science. B* 13(3):168–175. https://doi.org/10.1631/JZUS.B1100077

Rudolph, D.V., Antoinette, D.V. (2023). "Blood Cancer Statistics" *Leukemia and Lymphoma Society*. https://www.lls.org/facts-and-statistics/facts-and-statistics-overview#Leukemia

Shahid, S., Shahid, W., Shaheen, J. (2021). Circulating miR-146a expression as a non-invasive predictive biomarker for acute lymphoblastic leukemia. *Scientific Reports* 11(1):22783. https://www.nature.com/articles/s41598-021-02257-4

Shinde, J.G., Srivaramangai, R. (2023). The evaluation of denoising techniques on microscopic blood smear images of acute lymphoblastic leukemia (ALL). *Open Access Research Journal of Engineering and Technology* 4(2):24–35.

Starczynowski, D., Kuchenbauer, F., Wegrzyn, J., Rouhi, A., Petriv, O., Hansen, C.L. et al. (2011). MicroRNA-146a disrupts hematopoietic differentiation and survival. *Experimental Hematology* 39(2):167–178. https://www.sciencedirect.com/science/article/pii/S0301472X10004923

Swellam, M., El-Khazragy, N. (2016). Clinical impact of circulating microRNAs as blood-based marker in childhood acute lymphoblastic leukemia. *Tumor Biology* 37(8):10571–10576. https://doi.org/10.1007/s13277-016-4948-7

Tavakoli, F., Jaseb, K., Far, M.A.J., Soleimani, M., Khodadi, E., Saki, N. et al. (2016). Evaluation of microRNA-146a expression in acute lymphoblastic leukemia. *Frontiers in Biology* 11(1):53–58. https://doi.org/10.1007/s11515-016-1387-1

Vasilatou, D., Papageorgiou, S., Pappa, V., Papageorgiou, E., Dervenoulas, J. (2010). The role of microRNAs in normal and malignant hematopoiesis. *European Journal of Haematology* 84(1):1–16. https://doi.org/10.1111/j.1600-0609.2009.01348.x

Xiao, M., Li, J., Li, W., Wang, Y., Wu, F., Xi, Y. et al. (2017). MicroRNAs activate gene transcription epigenetically as an enhancer trigger. *RNA Biology* 14(10):1326–1334. https://doi.org/10.1080/15476286.2015.1112487

Xiong, Q., Yang, Y., Wang, H., Li, J., Wang, S., Li, Y. et al. (2014). Characterization of miRNomes in acute and chronic myeloid leukemia cell lines. *Genomics Proteomics Bioinformatics* 12(2):79–91. https://www.sciencedirect.com/science/article/pii/S1672022914000266

Zhai, P.F., Wang, F., Su, R., Lin, H.S., Jiang, C.L., Yang, G.H., Yu, J., Zhang, J.W. (2014). The regulatory roles of microRNA-146b-5p and its target platelet-derived growth factor receptor α (PDGFRA) in erythropoiesis and megakaryocytopoiesis. *The Journal of Biological Chemistry* 289(33): 22600–22613. https://doi.org/10.1074/jbc.M114.547380

Zhang, H., Luo, X-Q., Zhang, P., Huang, L-B., Zheng, Y-S., Wu, J. (2009). MicroRNA patterns associated with clinical prognostic parameters and CNS relapse prediction in pediatric acute leukemia. *PLoS One* 4(11):e7826. https://doi.org/10.1371/JOURNAL.PONE.0007826