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Original Article

Profiling of microRNAs by next-generation sequencing: Potential biomarkers for diffuse large B-cell lymphoma

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الملخص

أهداف البحث: سرطان الغدد الليمفاوية يحتل المرتبة الخامسة بين أنواع السرطان الشائعة في جميع أنحاء العالم. سرطان الجهاز اللمفاوي هو الذي ينشأ من الخلايا التائية أو البائية. ترتبط الأورام اللمفاوية ذات الخلايا البائية الكبيرة المنتشرة بالغالبية العظمى من الأورام اللمفاوية اللاهودجكينية. يمكن أن تؤثر الرناوات الدقيقة غير المشفرة بشكل كبير على التعبير الجيني. يمكن استهداف العديد من الجينات بواسطة واحدة من الرناوات الدقيقة، وبالتالي، تؤثر بشكل كبير على شبكات التعبير الجيني. يمكن أن تعمل الرناوات الدقيقة كجينات مسرطنة أو مثبطات للورم للتحكم في تطور الأورام اللمفاوية في الخلايا البائية الكبيرة. بحثت هذه الدراسة في دور الرناوات الدقيقة في مرضى سرطان الغدد الليمفاوية في الخلايا البائية الكبيرة المنتشرة باستخدام تسلسل الجيل التالي، والذي أظهر الحساسية والدقة والمتانة.

طريقة البحث: شملت الدراسة 7 مرضى و3 عناصر تحكم حضروا عيادة أمراض الدم والأورام. تم استخراج الرناوات الدقيقة من عينات الأنسجة الموجودة المضمنة بالفورمالين والمضمنة بالبرافين. تم استخدام تسلسل الجيل التالي (نظام إليومينا) لتسلسل العينات لتحديد ملامح الرناوات الدقيقة.

النتائج: أظهرت عينات المرضى العديد من الرناوات الدقيقة مير-هسا (1248، 3607، 21، 142، 1244، 182، 6516، 766، 1291، 4449، و181أ)، في حين أظهرت العينات من الأفراد الأصحاء مير-هسا 14248، 3607، 21، 142 و 877. من المعروف أن مير-هسا-877-3ب يستهدف جينات متعددة، وتتفاعل الرناوات الدقيقة مثل مير-هسا-877-3ب و مير-هسا-1291 و مير-هسا-181أ-5ب في الغالب مع الجينات المستهدفة.

الاستنتاجات: تشير ملفات تعريف الرناوات الدقيقة من الأنسجة المنتشرة التي تحتوي على الفورمالين والثابتة والمضمنة بالبرافين لمرضى سرطان الغدد الليمفاوية في الخلايا البائية الكبيرة إلى أن مستويات الرناوات الدقيقة يمكن أن تميز مرضى سرطان الغدد الليمفاوية في الخلايا البائية الكبيرة المنتشرة عند مقارنتها بالضوابط. لذلك يمكن أن تكون بمثابة علامة حيوية تشخيصية أو تنبؤية للأورام اللمفاوية ذات الخلايا البائية الكبيرة المنتشرة. يمكن أيضا أن تكون الجينات المتغيرة و الرناوات الدقيقة أهدافا علاجية محتملة.

الكلمات المفتاحية: سرطان الغدد الليمفاوية؛ سرطان الغدد الليمفاوية في الخلايا البائية الكبيرة المنتشرة؛ الرناوات الدقيقة؛ تسلسل الجيل التالي؛ الفورمالين ثابت البارافين الأنسجة

Abstract

Background: Lymphoma ranks fifth in prevalence among common cancer types worldwide. This lymphatic system cancer arises from T or B cells. Diffuse large B cell lymphomas (DLBCLs) are associated with most non-Hodgkin lymphomas. Non-coding microRNAs (miRNAs) greatly affect gene expression. A single miRNA can target numerous genes, thus largely influencing gene expression networks. MiRNAs can act as oncogenes or tumor suppressors in controlling DLBCL progression. This study investigated the roles of miRNAs in patients with DLBCL through next-generation

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sequencing, which was found to be sensitive, accurate, and robust.

Methods: The study involved seven patients with DLBCLs and three controls at a hematology-oncology clinic. MiRNA was extracted from existing formalin-fixed, paraffin-embedded (FFPE) tissue specimens. Illumina next-generation sequencing was used to sequence samples for miRNA profiling.

Results: Samples from patients showed expression of various hsa-mir miRNAs (1248, 3607, 21, 142, 1244, 182, 6516, 766, 1291, 4449, and 181a), whereas those from healthy individuals showed expression of hsa-mir 1248, 3607, 21, 142, and 877. Hsa-mir-877-3p is known to target multiple genes, and miRNAs such as hsa-mir-877-3p, hsa-mir-1291, and hsa-mir-181a-5p interact primarily with target genes.

Conclusions: MiRNA profiling in FFPE tissues from patients with DLBCL suggested that miRNA levels can distinguish patients with DLBCL from controls, and therefore may provide prognostic or diagnostic biomarkers for DLBCL. Altered genes and miRNAs may also be potential therapeutic targets.

Keywords: DLBCL; FFPE; Lymphoma; miRNA; Next-generation sequencing

Introduction

Lymphoma, arising from B or T cells, affects more than 1 million individuals and is the fifth most prevalent type of cancer globally. Lymphomas are diverse malignancies with varying prognosis and pathogenetic mechanisms. More than 100 types of lymphoma have been described by the World Health Organization, most of which are of B-cell origin.[1] Recent GLOBOCAN data for 2020 have indicated the diagnosis of 544,000 new non-Hodgkin lymphoma cases worldwide, representing 2.8% of globally diagnosed cancers, and 260,000 deaths.[2]

In KSA, a variable increase in lymphoma trends has been observed in a study at King Saud University Medical City in Riyadh, involving 422 patients (most younger than 60 years) with advanced-stage disease. Diffuse large B-cell lymphoma (DLBCL) was the most common lymphoma subtype, and the survival data were comparable to those from studies in Western countries.[3] Histological analysis of biopsied material remains the gold standard for diagnosing B-cell lymphoma. However, the procedure is costly and painful, and requires routine examination by an experienced pathologist.

DLBCL pathogenesis is a complicated process involving accumulation of multiple genetic lesions that change the structure and expression patterns of oncogenes, tumor suppressor genes, and other pathogenetically important molecules. MicroRNAs (miRNAs) are crucial for normal B-cell development and are often expressed aberrantly in B-cell lymphoma. MiRNA profiling is considered a promising tool that may reveal biomarkers for DLBCL diagnosis and prognosis.[4–7] Several studies have identified many miRNAs as potential biomarkers. However, larger studies are required to validate and integrate specific miRNAs into clinical practice.[7,8]

MiRNAs post-transcriptionally control gene expression by specifically binding the untranslated regions of messenger RNAs (mRNAs) at the 3′ end. This interaction, based on sequence complementarity, facilitates either mRNA degradation or translational inhibition.[9] A single miRNA can greatly affect gene expression networks by targeting several genes.[10] More than 30% of protein-coding genes in humans are considered miRNA-conserved targets.[9] Studying miRNA is more complicated than studying nucleic acids, because of miRNAs' short length, high sequence similarity among family members, and high repetition rate. Furthermore, primary and mature miRNA forms must also be distinguished.[11] In profiling samples through next-generation sequencing (NGS) technologies, technical replicates are unnecessary, because of the high technical reproducibility.[12]

MiRNAs contribute to the pathogenesis of DLBCL in several ways. MiRNAs regulate DLBCL cancer progression by serving as tumor suppressors and oncogenes. Numerous miRNA lymphoma biomarkers notably regulate the functioning of healthy lymphocytes. Dysregulation of these miRNAs can lead to lymphoma development, given that several miRNAs are key regulators of healthy B-cell development, and their abnormal expression results in the development of pathogenic factors for B-cell lymphomas.[13] Aberrantly expressed miRNAs have been identified as critical DLBCL pathogenic factors. Several miRNAs are known to participate in the control and emergence of other hematological cancers. B-cell development in healthy humans depends primarily on miRNAs. In vivo overexpression of miR-155 in a DLBCL mouse model has indicated its potential role as an oncogene.[14] In contrast, miR-144–based inhibition of *BCL6* activity in DLBCL xenografted mice has been reported, thus revealing its tumor-suppressing potential. Biologically, in vitro forced miR-144 expression has been shown to greatly decrease the ability of OCI-Ly3 cells to proliferate and invade their surroundings, and miR-144–based tumor suppression has been demonstrated in a xenograft mouse model.[15] Additionally, tumor microarray analyses and cell culture investigations have revealed the effects of miR-187 and miR-10a on *BCL6* expression, cell mortality, and tumor suppression. These miRNAs may potentially be used as novel therapeutic targets in DLBCL.[16,17] A study by Kozloski et al.[18] has demonstrated NF-signaling inhibition by miR-181a, wherein excessive expression of miR-181a decreases the activity and expression of important DLBCL NF-signaling molecules, thus decreasing the survival and proliferation of tumor cells by targeting the *REL, CARD11, RELA, NFKB1A,* and *NFKB1* genes. However, in another study, *AXL* mRNA and protein expression in OCI-LY10 cells has been found to increase after down-regulation of miR-34a. An important increase in OCI-LY10 cell proliferation, metastasis, and infiltration, and greatly decreased apoptosis, were observed at lower miR-34a levels.

Therefore, miR-34a may contribute to DLBCL etiology by controlling *AXL*, which may also be a novel therapeutic target.[19] MiRNAs can act as tumor suppressors by inhibiting long noncoding RNAs such as PD-L1, *NEAT1*, and *MALAT1*. A study of cell cultures and tissue from patients and unaffected controls has reported that upregulation of PD-L1 downregulates the DLBCL miR-214, thus demonstrating the antagonistic features of these genes.[20]

Many studies have indicated various ways which miRNAs can be beneficial as biomarkers. Multiple studies have used miRNAs in DLBCL diagnosis and differentiation from Burkitt lymphoma.[21,22] Additionally, only 5% of patients with DLBCL with poor prognosis experience CNS relapse. Pillar et al.[23] have found that those patients have high levels of miR-20a and miR-30d expression. Expression patterns may potentially be used to differentiate patients with DLBCL.

Recently, more than 51 differentially expressed miRNAs have been found in patients with DLBCL through NGS.[24] The development of digital count technologies has facilitated the application of new miRNA profiling techniques. Through NGS, novel miRNAs can be simultaneously identified and verified.[12] Herein, NGS was used to investigate the functions of miRNAs in Saudi patients with B-cell lymphoma. The use of NGS to discover and explore miRNA profiles is supported by its sensitivity, wide dynamic range, and consistent fold change prediction, as compared with those of the gold standard, qPCR. NGS does not require technical replicates, because of its high reproducibility,[12] and RNA can be extracted from properly treated and archived formalin-fixed, paraffin-embedded (FFPE) tissues. RNA from 10-year-old samples has shown a complete success rate of extraction with short primers in qRT-PCR, thus indicating that proper experimental design can overcome the effects of archiving time. NGS has displayed a higher success rate than qRT-PCR.[25] Furthermore, NGS-based miRNA analysis of FFPE tissue has exhibited high robustness, sensitivity, and accuracy for comprehensive miRNA profiling.[12] In this study, we aimed to investigate the roles of miRNAs in patients with DLBCL in our region, to identify potential biomarkers through NGS, which demonstrated sensitivity, accuracy, and robustness, given that our patient cohort might have different genetic variations from those in other patients worldwide.

Materials and Methods

Study site and recruitment of participants

In this retrospective study, patients and controls were recruited from one hematology oncology center in the KSA. Seven patients with DLBCL and three control participants visiting the hematology-oncology clinic were enrolled (Table 1). We took sections from existing FFPE tissue blocks from lymph node biopsies of patients with DLBCL. Written consent to participate was obtained from all participants. Ethical approval was obtained from the local biomedical ethics committee (Medical and Bioethics Unit at the Faculty of Medicine and University Hospital at King Abdul-Aziz University). Lymph node biopsies were identified, relevant medical data were recorded, and corresponding FFPE sections were sent to the Hematology Research Unit. Eight sections (with a thickness of 5 μm) were cut with a conventional rotary microtome. Only patients with confirmed DLBCL were selected for the study. Patients with other malignancies, infections, and chronic diseases were excluded.

FFPE RNA extraction

An miRNeasy FFPE Kit (Qiagen, Germany) was used for DNA, RNA, and miRNA purification from FFPE tissue. For isolation of RNA longer than 18 nucleotides, the kit was able to recover miRNAs and other small RNAs for RNA sequencing and RT-PCR. The kit was also able to efficiently reverse formaldehyde modification without damaging the RNA. Therefore, nucleic acid purification from FFPE samples was effectively performed while DNA contaminants were removed through the DNase digestion step.

Initially, xylene (1 ml) was used to remove paraffin from the FFPE tissue. Subsequently, a lysis buffer containing Proteinase K was used to incubate the samples to release RNA. Samples were then subjected to a short incubation at a high temperature, to reverse formalin crosslinking of released RNA, and increase quality and yield. Subsequently, DNase treatment was performed to completely remove genomic DNA fragments.

After addition of RBC buffer, followed by ethanol for better RNA binding, the lysates were subjected to RNeasy MinElute (Qiagen, Germany) spin column purification, in which miRNA (including total RNA) binds the membrane, and contaminants are washed away. RNase-free water was used to elute the total RNA. Ethanol (1 ml of 96−100%) was added to the RNA pellet for removal of xylene residues. The RNA pellet was then resuspended in PKD buffer (240 μL) and Proteinase K (10 μL). Sample incubation was performed for 15 min at 56 °C, then another 15 min at 80 °C.

Next, DNase Booster Buffer (25 μL) and DNase I stock solution (10 μL) were added, and incubation was performed for 15 min at room temperature. Subsequently, RBC buffer (400 μL) and ethanol (1400 μl of 100%) were added. The entire volume was transferred to an RNeasy MinElute spin column inside a 2-ml collection tube and washed twice with RPE buffer. Finally, RNA was eluted by the addition of RNase-free water (20 μl). The integrity and purity of total RNA and miRNA were assessed with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., USA). Samples were then stored at −80 °C until analysis.

RNA quality control

The assessment of RNA quality, quantity, and integrity was performed by fluorometry with a Qubit RNA HS Assay Kit (Invitrogen Qubit 4). Briefly, two standards were required for the Qubit RNA HS assay. The Qubit working solution was prepared by dilution of Qubit RNA HS reagent in Qubit RNA HS buffer at a dilution ratio of 1:200. The working solution was transferred to a 200 μL tube. The Qubit standards (10 μL each) were added to a tube along with 1 μL of each sample. Incubation was performed at room temperature for 2 min, and the tubes were subjected to fluorometry.

Sequencing of miRNA

Next generation sequencing, which can discover and verify novel miRNAs, is a new method for lymphoma diagnosis, prognosis, and development of treatment strategies. A TruSeq Small RNA Library Prep Kit (Illumina, USA) was used to prepare purified small RNA. Adapters were ligated to the RNA molecule ends, and reverse transcription and amplification were performed to generate a complementary DNA (cDNA) library. Gel purification was then performed to prepare the library for clustering and sequencing. Ten unique indexes were used for the multiplex sequencing and analysis to discover small RNA and miRNA molecules (Figure 1).

A TruSeq Small RNA Library Prep Kit (Illumina, USA) was used to extract the total RNA (1 μg) in nuclease-free water (5 μL) to ligate the adapters at the 3′ and 5′ ends. Reverse transcription and amplification generated the cDNA constructs based on the small ligated RNA. Adapters (at the 3′ and 5′ ends) containing RNA fragments were selectively enriched during this step by using two primers. Subsequently, gel purification of the amplified cDNA construct was performed for cluster generation. The band of 136–143 base pairs (bp) contained primarily mature miRNA, which was generated from small RNA fragments of approximately 22 bp. The band of 150–155 bp contained small RNA and pre-miRNAs, which were generated from RNA fragments of approximately 30 bp. A high-sensitivity DNA-specific chip was used for the quality control analysis of the library with a Bio-analyzer (Agilent Technologies 2100). The concentration of the library was normalized to 2 nM with Tris–HCl buffer (10 mM). Finally, a NextSeq 550 system was used for sequencing with a NextSeq 500/550 kit v2.5 (75-cycle) reagent cartridge (Illumina, California, U.S.).

Sequencing data analysis

Multiple software programs from Illumina (https://www.illumina.com) were used to analyze small RNA sequencing, including BaseSpace Small RNA application (version 1.0.1). Novel precursors and mature miRNAs were predicted with miRDeep (version 3.2), whereas miRNA information was obtained with miRBase (version 21). A read was required to be aligned on the same strand at the start of a reference sequence of the same length. Data for mature miRNAs were used for pre-miRNA grouping. Relative frequency was used to assess expression changes through comparison of normalized read counts between samples. The top five sequences of each sample with the most reads were listed, whereas reads with low numbers were excluded. The relative frequency predicted mature miRNAs.

Prediction of miRNA targets

Gene target prediction was performed with the online bioinformatics tool miRWalk (version 3) http://mirwalk.umm.uni-heidelberg.de/. The Target Mining page of miRWalk was used to search for various gene and miRNA targets. A list of miRNAs in both groups (patients and controls), containing names of predicted mature miRNAs, was provided. The list of DLBCL-associated genes was filtered according to Kyoto Encyclopedia of Genes and Genomes annotations.[26]

Statistical analysis

DATAtab Statistics Calculator (2023) (https://datatab.net) was used to prepare charts. Chord (cross-talk) diagrams were generated in PlotAPI (2023) (https://plotapi.com). Diagrams and illustrations were created in BioRender (www.biorender.com, 2023). GraphPad Prism v9.4.1 (https://www.graphpad.com) was used to visualize relative miRNA frequency in heatmaps.

Results

miRNA sequencing

The quality of small RNA was assessed, and results were obtained for all ten samples (seven patients and three controls). Novel or known miRNAs were not predicted in any samples. A known number of pre-miRNAs was observed in all samples. The pre-miRNAs identified in patients included hsa-mir-1248, hsa-mir-3607, hsa-mir-21, hsa-mir-142, hsa-mir-1244, hsa-mir-182, hsa-mir-6516, hsa-mir-766, hsa-mir-1291, hsa-mir-4449, and hsa-mir-181a, whereas those identified in healthy controls included hsa-mir-1248, hsa-mir-3607, hsa-mir-21, hsa-mir-142, and hsa-mir-877. Some Pre-miRNAs were found in both groups, including hsa-mir-1248, hsa-mir-3607, hsa-mir-21, and hsa-mir-142 (Figure 2).

The relative frequency of readings was calculated to reflect miRNA expression. A comparison between patient and control samples revealed differences in expression (Figure 3). Comparison of the two groups with a box-and-whisker plot of relative frequency demonstrated a twofold difference in mean hsa-mir-1248 and hsa-mir-3607, whereas the means of hsa-mir-21 and hsa-mir-142 expression were convergent (Figure 4).

Gene targeting

Interactions were identified among 15 expressed miRNAs, and 18 experimentally validated altered genes in DLBCL were obtained from miRwalk. The *TP53* gene was the most targeted gene by hsa-mir-21-3p, hsa-mir-6516-5p, hsa-mir-1291, and hsa-mir-181a-5p, whereas the miRNA hsa-mir-877-3p targeted a wide range of genes (Figure 5).

Table 1: Patient characteristics.

Characteristic	Patients (n = 7)
Median age, years	55 (45–64)
Sex (male/female)	5/2
Mean LDH level (U/L)	850 (189–3162)
Ann Arbor staging	
Limited (stage I/II)	1 (15%)
Advanced (stage III/IV)	6 (85%)
Cell of origin	
Germinal center	4 (57%)
Non-germinal center	3 (43%)

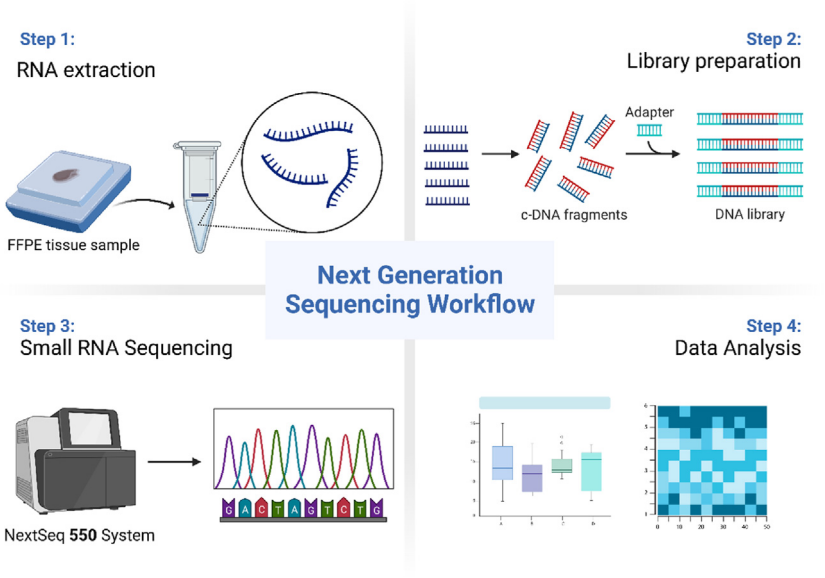

Figure 1: Next generation sequencing workflow (created with BioRender.com).

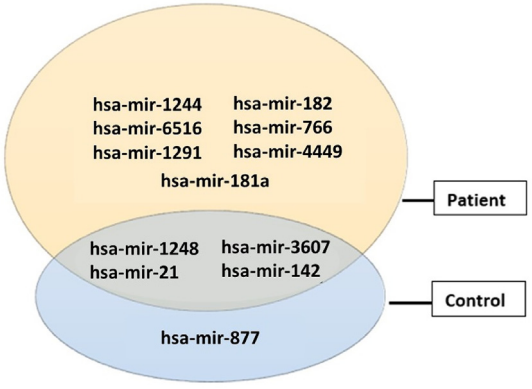

Figure 2: Venn diagram of overlapping miRNAs between patients and controls (n = 10). RNA profiling was performed with next-generation sequencing (Illumina system).

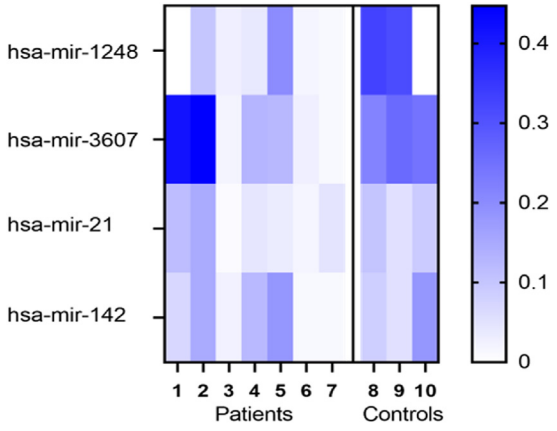

Figure 3: Heatmap showing predicted expression of miRNAs in patients and controls, suggesting that different miRNA regulation may be associated with DLBCL prognosis.

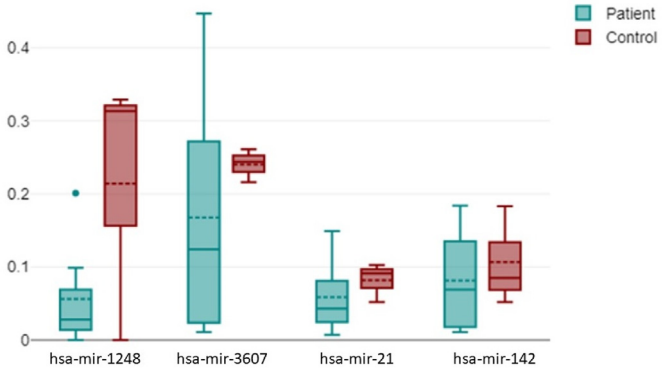

Figure 4: Box-and-whisker plot of the relative frequency of shared miRNAs; hsa-mir-1248 was three times higher in controls than patients.

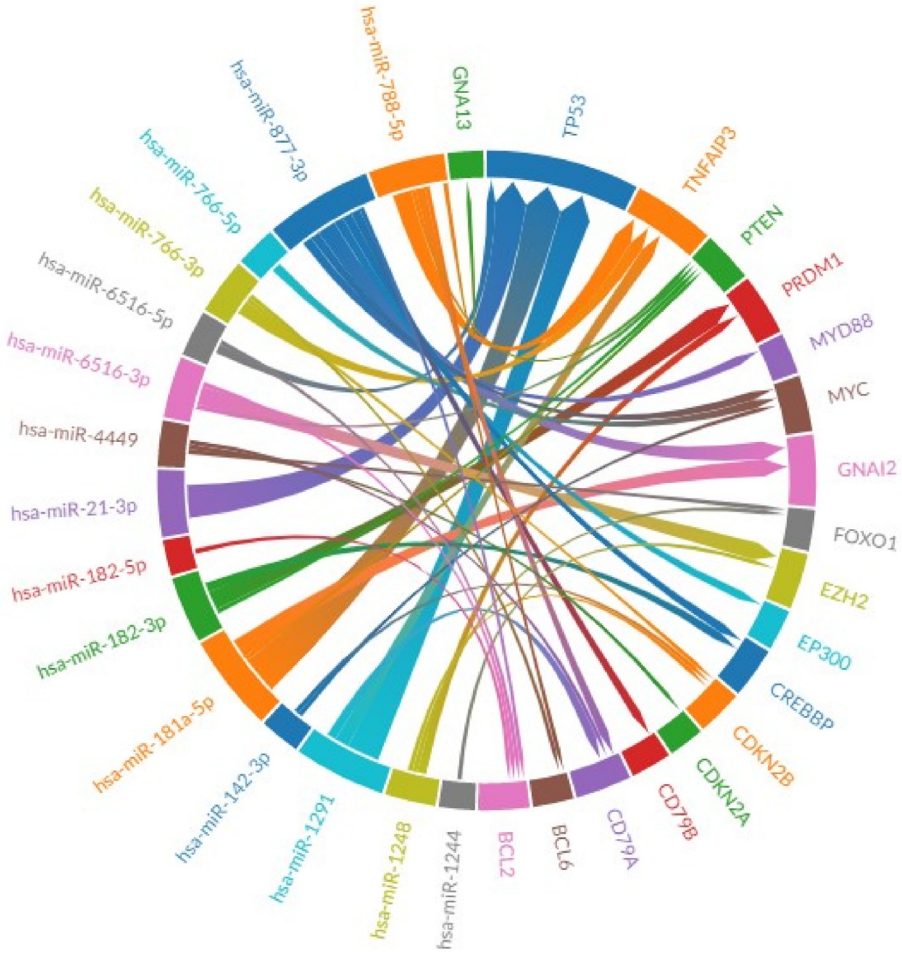

Figure 5: Chord diagram of predicted miRNA interactions with host genes. Data from KEGG[26] and miRWalk (http://mirwalk.umm.uni-heidelberg.de) were used for the analysis, representing the interactions of differentially expressed miRNAs and altered genes in DLBCL. The sizes of rectangles next to the names of target genes and miRNAs represent the proportional number of interactions.

Discussion

RNA can be extracted from archived FFPE tissue samples for NGS, if samples are properly treated before fixation. RNA from 10-year-old samples has been successfully used in qRT-PCR with short primers, thus indicating that archiving time effects can be avoided through the use of a proper experimental design. The success rate of NGS is higher than that of qRT-PCR.[25] MiRNA analysis of FFPE tissue has demonstrated the high accuracy, robustness, and sensitivity

of NGS for detailed miRNA profiling.[12] Archived FFPE tissue samples can be effectively used in retrospective studies. In this study, existing FFPE samples from participating patients, collected between 2016 and 2021, were used, because obtaining fresh samples was not possible.

Sample quality and preservation methods are important criteria in FFPE tissue studies. This study extensively investigated FFPE tissue samples and obtained favorable results. The data revealed large differences in miRNA expression between patients and healthy controls. The presence of hsa-mir-1248, hsa-mir-3607, hsa-mir-21, hsa-mir-142, hsa-mir-182, hsa-mir-1244, hsa-mir-6516, hsa-mir-766, hsa-mir-1291, hsa-mir-4449, and hsa-mir-181a was observed in the patients, whereas hsa-mir-1248, hsa-mir-3607, hsa-mir-21, hsa-mir-142, and hsa-mir-877 were found in the healthy controls. Some miRNAs, including hsa-mir-1248, hsa-mir-3607, hsa-mir-21, and hsa-mir-142, overlapped between patients and healthy controls. RNA extraction and library preparation techniques can greatly influence a sample's fragment composition.[27] MiRNA expression, reflecting relative frequency, differed among patients, controls, and DLBCL subtypes. However, analysis of a larger number of samples would be required to assess statistical significance. The presence of miRNAs and their expression levels may potentially aid in DLBCL diagnosis and prognosis. The interaction between the identified miRNAs and experimentally validated DLBCL-altered genes demonstrated that *TP53* was the most targeted gene by hsa-mir-21-3p, hsa-mir-6516-5p, hsa-mir-1291, and hsa-mir-181a-5p, whereas hsa-mir-877-3p miRNA targeted a wide range of genes. The miRNAs interacting primarily with targeted genes included hsa-mir-877-3p, hsa-mir-1291, and hsa-mir-181a-5p. This interaction between expressed miRNAs and altered genes revealed potential miRNA target genes that may be focused on during therapy. Although our study is the first to explore miRNAs in the Saudi DLBCL population, we provide a comprehensive discussion incorporating findings from other population studies on DLBCL and related cancers. This approach contextualizes our results and contributes to broader understanding of the involvement of miRNA in DLBCL across diverse populations.

Some genes have been reported to be associated with lymphoma, whereas others have been associated with other cancerous diseases. During this study, relationships of hsa-miR-21 and hsa-miR-181a with DLBCL were demonstrated by targeting specific tumor suppressors (PTEN, PDCD4, and FOXO1). MiR-21 functions as an oncogene by activating the PI3K/AKT/mTOR oncogenic pathway.[28] extremely elevated serum miR-21 expression has been reported in individuals with DLBCL compared with healthy controls. Elevated miR-21 expression significantly correlates with B symptoms (fever, night sweat, or weight loss).[29] Bcl-2 and miR-21 may serve as useful targets for future DLBCL treatments, because they enhance viability and decrease DLBCL cell apoptosis.[30] Additionally, miR-21 reacts to chemotherapy administered to patients with DLBCL.[31] The expression of miR-181a is lower in ABC-like DLBCL cells than GCB-like DLBCL cells. Overexpression of miR-181a increases apoptosis, decreases invasiveness, and causes G0/G1 cell cycle arrest in ABC-like DLBCL cells.[32] One study has reported diminished activity and expression of essential NF-signaling subunits in DLBCL with excessive expression of miR-181a, thus further inhibiting tumor cell growth.[18] An association of hsa-mir-3607 and hsa-mir-6516 with lymphoma or other cancerous diseases was not found during this study; however, hsa-mir-1248, hsa-mir-766-3p, and hsa-mir-1291 may be potential tumor suppressor genes in colorectal cancer.[33–35] In addition, the upregulation of miRNA-766-5p is known to inhibit migration, invasion, and cell growth in prostate cancer.[36] Similarly, we observed a high frequency of hsa-mir-1248 in the healthy controls, thus indicating a tumor suppressor role in DLBCL. Therefore, this miRNA might be used as a therapeutic target. A significant correlation between hsa-mir-4449 and the development of hepatocellular carcinoma and gastric cancer has been reported,[37,38] whereas miR-142 has tumor-suppressing properties against hepatocellular, colon, lung, and breast carcinoma.[39] Downregulation of hsa-mir-877 in an osteosarcoma cell line and tissues has been observed.[40] Lower levels of miR-877-5p have been reported in patients with gastric cancer than controls; moreover, overexpression of this miRNA greatly inhibits cell cycle progression and cell growth.[41] Similarly, hsa-mir-877 exhibits low expression in ovarian cell lines and cancer tissues.[42] Diminished expression of miR-877-5p has been observed in prostate cancer tissues and cells, whereas its overexpression has been reported to have tumor-suppressing effects.[43]

In this study, the identification of hsa-mir-877 in healthy controls indicated its tumor suppressor role, in agreement with previous findings. However, further investigations involving larger case series should be conducted to validate the findings of this study, and define the roles of miRNAs as potential biomarkers for diagnosis and prognosis. Validation of miRNAs as therapeutic targets is also necessary. After successful clinical trials, miRNAs as therapeutic targets, combined with administration of conventional drugs, may provide a preferred choice for DLBCL treatments in the future.

Conclusions

MiRNAs are noncoding RNA molecules that regulate RNA silencing and post-transcriptional gene expression. MiRNAs act as tumor suppressors or oncogenes in controlling DLBCL malignancy. FFPE tissues are a valuable resource in clinical research. Generally, the nucleic acids from FFPE tissues are chemically altered and fragmented, thus complicating their use in molecular studies. DNA and RNA, if they are carefully processed before fixation, can be extracted from FFPE tissues for advanced molecular assays. Multiple sample preparation steps can potentially introduce biases that may lead to sequencing errors. Furthermore, this study performed NGS to investigate the roles of miRNAs in Saudi patients with B-cell lymphoma. MiRNA profiling from FFPE samples indicated differential levels between patients with DLBCL and controls, thereby suggesting that miRNA levels may serve as diagnostic or prognostic biomarkers for distinguishing patients with DLBCL from

healthy individuals. The interaction between identified miRNAs and altered genes may also make them potential therapeutic targets.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

Obtained on 01/09/2020, reference No 450-20.

Authors contribution

SB conceived and designed the study, collected data, and wrote the initial and final drafts of the manuscript. MA conducted the research and organized the data. AB wrote the final draft of the article. HA performed the laboratory work. HMA provided logistic support and wrote the final draft of the article. AA provided logistic support and wrote the final draft of the article. OR provided logistic support and wrote the final draft of the article. YD provided research materials. SI conducted the research. HAA conceived and designed the study. IQ conceived and designed the study. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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