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Regular paper

Serum miR-21 predicts the prognosis of patients with primary gastrointestinal diffuse large B-cell lymphoma

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Background: Primary gastrointestinal diffuse large B-cell lymphoma (PGI-DLBCL) lacks specific clinical manifestations and its malignancy renders prognostication and choice of treatment strategy difficult. The aim of this study was to evaluate microRNA (miR)-21 as potential non-invasive biomarkers for prognosis in PGI-DLBCL patients. Methods: Serum miR-21 expression in de novo PGI-DLBCL patients, consecutively enrolled for this study, was detected by quantitative real-time polymerase chain reaction (qRT-PCR). Relative expression was calculated using the comparative Ct method. Statistical significance was determined using the Mann-Whitney rank sum and Fisher's exact test. Survival analysis was conducted using the Kaplan-Meier method. Results: Compared with healthy controls, serum miR-21 levels were significantly elevated in the PGI-DLBCL patients (n=156). The high expression level of serum miR-21 at diagnosis was associated with worse progression-free survival (PFS) (30 (9–42) *vs* 42 (12–52) months in high and low miR-21 groups) and overall survival (OS) (35 (15–52) *vs* 48 (17–61) months in high and low miR-21 groups) and was an independent risk factor for PFS and OS (hazard ratios 4.345 and 3.311, respectively). Furthermore, Bcl-2, Bcl-6 and Ki-67 were independently and positively associated with miR-21 expression. Conclusions: Our results suggest that miR-21 is a potential prognostic marker to predict clinical outcomes in PGI-DLBCL patients and a high miR-21 level is associated with poor outcomes.

Keywords: PGI-DLBCL, biomarkers, miR-21, prognosis, therapeutic outcomes

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Abbreviations: ANOVA, analysis of variance; Bcl-2, B cell lymphoma-2; Bcl-6, B cell lymphoma-6; CRP, c-reactive protein; ECOG, East-ern Cooperative Oncology Group; FFPE, formalin-fixed and paraffin-embedded; FISH, fluorescent in situ hybridization; GC, germinal center; HR, hazard ratio; IHC, immunohistochemistry; Lnc, long non-coding; miR, microRNA; NHL, non-Hodgkin's lymphoma; non-GC, non-germinal center; OR, odds ratio; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1,programmed cell death ligand 1; PFS, progression-free survival; PGI-DLBCL, primary gastrointestinal diffuse large B-cell lymphoma; PTEN, phosphatase and tensin homologue; qRT-PCR, quantitative real-time polymerase chain reaction; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; SHIP1, Src homolo-gy-2 domain-containing inositol 5-phosphatase 1

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma (NHL), counting for 30–40% of new hematological malignancies in different geographic regions (Li *et al*., 2018). Primary gastrointestinal diffuse large B-cell lymphoma (PGI-DLBCL) occurs mostly in extranodal sites such as the stomach (60–70%), followed by the small bowel, ileum, cecum, colon and rectum (d'Amore *et al*., 1994). It lacks specific clinical manifestations and has complex pathological features, resulting in difficult diagnosis and treatment (Ferreri & Montalban, 2007; Ruskone-Fourmestraux *et al*., 2003). Therefore, accurate prognostication is needed to help to design the initial treatment plan (Cabanillas & Shah, 2017; Nikonova *et al*., 2015). DLBCL has typical invasiveness and could be cured with chemotherapy and targeted therapy (Fu *et al*., 2008; Salles *et al*., 2020). However, the relationship between DLBCL subtypes based on the traditional morphological classification and therapeutic efficacy and prognosis is not fully clear. Advances in epigenetics have been made toward the biological diversity and signaling pathways involved in tumorigenesis. For example, microRNAs (miR) miR-155-5p and miR-21-5p have been found to be potentially useful for diagnosis (Larrabeiti-Etxebarria *et al*., 2019), long non-coding (lnc) RNA SNHG14/miR-5590-3p/ZEB1 are shown to form a positive feedback loop that promotes progression and immune evasion of DLBCL through regulating PD-1/PD-L1 checkpoint (Zhao *et al*., 2019), and circulating miRNAs are revealed to be capable of predicting relapse and survival in DLBCL patients (Sun *et al*., 2021).

MiR-21, as one of the most highly expressed members of the small non-coding miRNA family in many mammalian cell types (Lagos-Quintana *et al*., 2001), is considered to play key roles in many cellular processes, such as functioning as a key switch in the inflammatory response (Sheedy, 2015), promoting myocardial infarction-induced cardiac fibrosis (Yuan *et al*., 2017), affecting tumorigenesis by downregulating the expression of target genes (Liu *et al*., 2019; Song *et al*., 2014). It is also demonstrated as a promising biomarker for the early detection of colon cancer, especially in men (Dehghan *et al*., 2019), breast cancer (Anwar *et al*., 2019) and hepatocellular carcinoma (Guo *et al*., 2017). Furthermore, miR-21 and miR-155 overexpression may activate the AKT signaling pathway and down-regulation of tumor suppressors such as phosphatase and tensin homologue (PTEN), programmed cell death 4 (PDCD4), or Src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP1) in natural killer-cell lymphoma/leukemia, leading to decreased apoptosis and increased proliferation of tumor cells (Yamanaka *et al*., 2009). High expression of miR-21 in malignant tissue and blood has been associated with DLBCL diagnosis (Chen *et al*., 2014; Lawrie *et al*., 2008). However, very

few reports to date have evaluated the role of circulating miR-21 as a potential prognostic factor in DLBCL (Li *et al*., 2015) and whether it can be used as a prognostic marker in PGI-DLBCL is still unclear.

The goal of this study is to investigate the potential of serum miR-21 for prognostication of PGI-DLBCL to assist the management of the disease.

MATERIALS AND METHODS

Study design, patients, and control subjects

This was a prospective, observational, non-interventional study on a cohort of newlydiagnosed de novo PGI-DLBCL adult patients consecutively enrolled at Zunyi Medical University between March 2014 and March 2018. All patients were treated with six courses of R-CHOP every 21 days (rituximab 375 mg/m^2 day 1, cyclophosphamide 750 mg/m^2 day 1, vincristine 1.4 mg/m^2 day 1, doxorubicin 50 mg/m^2 day 1, prednisone 100 mg days 1–5), followed by two adjunctive doses of rituximab (Khan *et al*., 2016; Shibata *et al*., 2017). Patients were included if he/she was equal to or older than 18 years, and histopathologically proven to have PGI-DLBCL. Patients were excluded because of comorbidity with other malignant tumors, blood system diseases and other life-threatening conditions that could compromise clinical outcomes.

This study was approved by the Ethics Committee of Zunyi Medical University, Zunyi, China, and all study participants provided written informed consent. All patients were treated according to ethical and legal standards adopted by the Declaration of Helsinki. Histological criteria for diagnosis and classification of DLBCL are those of the World Health Organization (WHO) classification (Swerdlow *et al*., 2016). Age- and gender-matched healthy controls were recruited at the same hospital from individuals performing a routine health checkup.

Assessments

Clinical assessment was performed by Ann Arbor stage and IPI evaluation (Ruppert *et al*., 2020)and the Eastern Cooperative Oncology Group (ECOG) performance status was assessed as previously (Prigerson *et al*., 2015). The cell of origin was determined by IHC using the Hans algorithm as germinalcenter (GC) or non- germinal center (non-GC) immunophenotype (Hans *et al*., 2004). Formalin-fixed and paraffin-embedded (FFPE) tumor sections were analyzed for Bcl-2, Bcl-6, c-Myc and ki67 expressions by interphase fluorescent in situ hybridization (FISH) as previously reported (Yan *et al*., 2014). Briefly, paraffin-embedded tissue slices were rehydrated by going through an ethanol serial and hybridized to fluorescent probes at room temperature for 2 h according to the supplier's instructions. The probes for Bcl-2, Bcl-6, c-Myc and ki67 probes were purchased from Beyetime, Beijing. FISH signals were analyzed using a fluorescence microscope (Olympus BX51, Tokyo, Japan) equipped with a DP72 camera and DP2-BSW software (Olympus, Tokyo, Japan). Patient cases with break-apart signals in >10% of nuclei were considered positive for the presence of a translocation. The signal distributions were evaluated by two independent observers who were blinded to the patient information.

Baseline disease staging was determined according to the Lugano recommendations for non-Hodgkin lymphoma (Cheson *et al*., 2014). Symptoms such as drenching night sweats, fever (>38°C, lasting for more than 3 days), and unexplained loss of more than 10% body weight within 6 months, were recorded.

Data collection and follow-up

Demographic and clinical data were collected from the hospital medical databases, including age, gender, medications, and laboratory findings. All patients were followed up by monthly telephone interviews, during hospital visits and hospital readmission till July 2020. Overall survival (OS) was defined as the time interval between the date of initial diagnosis and the date of death from any cause, and progression-free survival (PFS) was defined as the time interval between the date of initial diagnosis and the date of disease progression or death from any cause, whichever occurred first.

RNA isolation from blood samples, cDNA synthesis and qRT-PCR

Five ml fasting blood samples were collected in the morning within 2 days following the diagnosis in tubes containing dipotassium ethylenediaminetetraacetic acid (EDTA K2**)** and centrifugated at 500×*g* for 10 min to collect serum. 500 µl was used for RNA extraction using Trizol reagents (CW0580S, Cwbiotech, Beijing) according to the supplier's instructions and was reversely transcripted to cDNA using miRNA cDNA Synthesis kit (Cat. no: A28007, Thermo Fisher Scientific**,** USA) according to manufacturer's protocols. HiScript II qRT SuperMix for qPCR (cat. no: R222-01, Vazyme, USA) was used to amplify the cDNA according to the manufacturer's protocols. Normalization was done with U6 (forward: GCTTCGGCAGCACATATACTAAAAT, reverse: CGCTTCACGAATTTGCGTGTCAT). The PCR was performed in a total volume of 10 µl containing 1.5 µl of diluted and pre-amplified cDNA, 5 µl of 2×SYBR Green PCR Master Mix and 1 µl of each fluorescence probe. The cycling conditions were 50ºC for 2 min, 95ºC for 10 min followed by 45 cycles, each one consisting of 10 s at 95ºC and 30 s at 58ºC. Samples were run in triplicate and the mean value was calculated for each case. The data were managed according to the previously described protocol (Livak & Schmittgen, 2001). The primer sequences used for miR-21 were forward: 5'-GTGCAGGGTCCGACGGT. reverse: 5'-GCCGCTAGCTTATCAGACTGATG.

Statistical analysis

Descriptive statistics were calculated for all the variables of interest to summarize the patient's characteristics. Statistical software (SPSS22.0) was used for statistical analysis. Measurement data with normal distribution were expressed as mean ±S.D. (standard derivation) and non-normal distribution data were expressed as a median. Counting data were expressed as a percentage. Analysis of variance (ANOVA) was used to compare normallydistributed variables, the Kruskal-Wallis test was employed for non-normally distributed variables, and the chi-square of Fisher's exact test, as appropriate, was used for categorical data. Spearman rank correlation analysis was used to evaluate the relationship between serum miR-21 and non-normal variables. The Kaplan-Meier survival curve was used to display the survival times, which were compared using the log-rank test. Univariate and multivariate Cox regression models were used to analyze the relationship between serum miR-21 and clinicopathological factors, computed with the R package

smoothHR as previously described (Meira-Machado *et al.*, 2013). A value of $P<0.05$ was considered statistically significant.

RESULTS

Patient characteristics

From March 2014 to March 2018, serum samples were collected from 176 newly diagnosed PGI-DLBCL patients who completed R-CHOP based treatment, 20 cases who did not complete the treatment courses were excluded in the present analysis (including 8 toxic deaths during treatment and 12 cases of loss of follow-up). Baseline clinical and biological features of 156 cases are shown in Table 1. The median age of the patients was 59.5 years, including 88 male and 68 female. The Ann Arbor stages of the patients ranged from I to IV with most of them being at stage III–IV. The IPI ranged from 0 to 5 and lactate dehydrogenase level was normal in half of the patients. Most of the cells of origin were GC and serum CRP level was elevated in about 50 % of patients. The proportion of Bcl-2, Bcl-6 and c-MYC positive cells was between 74% to 85 % (Table 1).

Table 1. General characteristics and baseline clinicopat hological features of PGI-DLBCL patients

Variables	n	%	*P* values
Age, median (range)	59.5 (22–79)		
Gender, n (%)			0.834
Male	88	56.4	
Female	68	43.6	
Ann Arbor stages, n (%)			0.024
I–II	47	30.1	
III–IV	109	69.9	
IPI, n (%)			0.023
0–1	56	35.9	
2–3	45	28.8	
4–5	55	35.3	
Lactate dehydrogenase, n (%)			0.856
Normal	72	46.2	
High	84	53.8	
Ki-67, n (%)			0.786
<70%	66	42.3	
≥70%	90	57.7	
Bcl-2, n (%)			0.022
<50%	22	14.1	
≥50 %	134	85.9	
Bcl-6, n (%)			0.013
<30%	34	21.8	
≥30 %	122	78.2	
c-MYC, n (%)			0.012
<30%	40	25.6	
≥30 %	116	74.4	
Cell of origin, n (%)			0.012
Germinal center	123	78.8	
Non-germinal center	33	21.2	
Serum CRP, n (%)			0.565
Normal	68	43.6	
High	88	56.4	

IPI, Intonational prognostic index; CRP, C-reactive protein

Serum miR-21 level was elevated in PGI-DLBCL patients

We first determined miR-21 levels in the 156 PGI-DLBCL patients and 52 healthy controls using qRT-PCR, and the results showed that the levels were significantly elevated in PGI-DLBCL patients as compared with healthy control ($P<0.05$, Fig. 1).

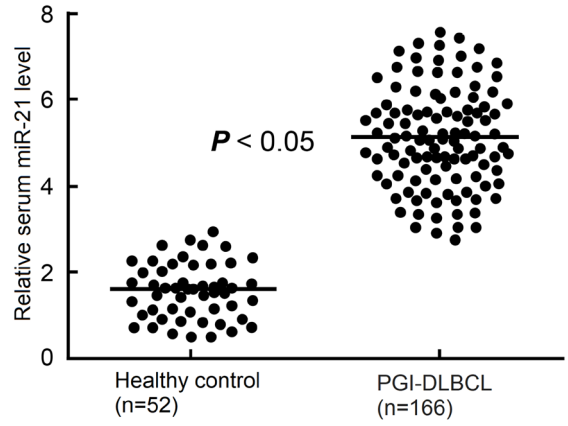

Figure 1. Expression levels of serum miR-21 in a cohort of PGI-DLBCL patients and healthy controls.
Relative expression was calculated using the comparative ΔCt method. ($P<0.05$, *P*-value was determined using the Mann–Whitney rank-sum test).

High miR-21 level was associated with poor outcomes

Since the relative miR-21 levels were highly variable in PGI-DLBCL patients, we stratified the patients into high (>5.2) and low (≤5.2) miR-21 level groups at the medium value (5.2) and analyzed their relationship with OS and PFS using the Kaplan-Meier survival curves. The median OS and PFS were 35 (15–52) and 30 (9–42) months in the high miR-21 level group and 48 (17–61) and 42 (12–52) months in the low miR-21 level group, respectively. The accumulated OS and PFS in the high miR-21 level group were significantly lower than those in the low miR-21 level group ($P<0.01$, Fig. 2).

With Cox regression analysis, miR-22 level, Bcl-2, Bcl-6 and Ki-67 expressions were found to be the factors independently affecting the probability of PFS and OS in our cohort of patients. For PFS, the hazard ratios were 4.345, 2.896, 2.104 and 2.779, and for OS, the hazard ratios were 3.311, 2.236, 2.214 and 2.169, respectively, while other factors such as Ann Arbor stage, IPI and Cell of origin were not significantly related to PFS and OS (Table 2).

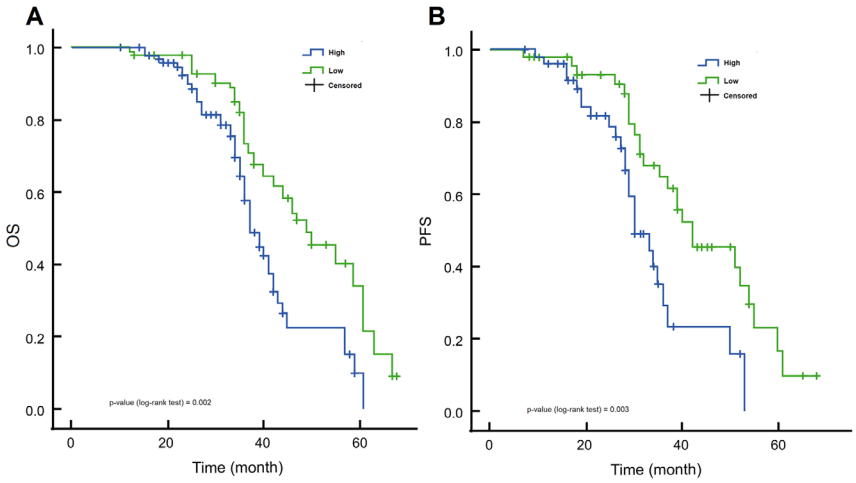

Figure 2. Kaplan-Meier PFS and OS curves of patients with high (above median) and low (below median) miR-21 levels.
PFS, progression-free survival; OS, overall survival

Table 2. Cox regression analysis of factors affecting progression-free survival (PFS) and overall survival (OS) in PGI-DLBCL patients

	PFS			OS		
Factors	HR	95% *CI*	*P*	HR	95% *CI*	*P*
miR-21, above vs below median value	4.345	2.123–8.123	0.017	3.311	1.123–4.343	0.016
Ann Arbor stage, I–II *vs* III–IV	1.126	0.238–2.104	0.058	1.021	0.228–2.901	0.068
IPI, 0–1 *vs* ≥2	1.105	0.271–2.539	0.055	0.905	0.211–1.839	0.075
Bcl-2, <50% *vs* ≥50%	2.896	1.612–5.761	0.028	2.236	1.512–4.751	0.037
Bcl-6, <30% *vs* ≥30%	2.104	1.331–4.175	0.011	2.214	1.551–4.545	0.012
c-MYC, <40% *vs* ≥40%	1.124	0.202–2.745	0.273	1.114	0.212–2.345	0.295
Ki-67, <70% *vs* ≥70%	2.779	0.942–3.543	0.021	2.169	0.911–3.123	0.021
Cell of origin, non-GC *vs* GC	0.826	0.23–2.38	0.253	0.811	0.212–2.454	0.213

HR, hazard ratio; CI, confidence intervals; IPI, International Prognostic Index; GC, germinal center; non-GC, non germinal center

Factors affecting miR-21 levels

Correlation analysis between miR-21 levels and clinicopathological characteristics and cancer-related genes showed that miR-21 level was closely associated with Ann Arbor stages and IPI grades, Bcl-2, Bcl-6, c-MYC and Ki-67 expressions, while other factors such as age, gender, lactate dehydrogenase, cell origin and serum CRP were not significantly related to miR-21 levels (Table 3) and logistic regression analysis showed that Bcl-2, Bcl-6 and Ki-67 were independently and positively associated with miR-21 expression (Table 4, $P<0.05$), but not with Ann Arbor stages, IPI grades or c-MYC.

DISCUSSION

MiR-21 is located on human chromosome 17q23.2 and plays a crucial role in a variety of biological functions and diseases including development, cancer, cardiovascular diseases, and inflammation (Kumarswamy *et al.*, 2011). Our study showed that serum miR-21 expression is elevated in PGI-DLBCL patients, high miR-21 level is associated with poor therapeutic outcomes and related to Bcl-2, Bcl-6, and Ki-67 expressions. These findings demonstrate that miR-21 has prognostic value for PGI-DLBCL and could be used to assess the therapeutic progression and outcomes for PGI-DLBCL patients.

Mature miR-21 is processed from primiR-21 (primary transcript containing miR-21) located within the intronic region of the TMEM49 gene. Expression of miR-21 has been found upregulated in many types of cancers including DLBCL (Lawrie *et al.*, 2007, 2008) and miR-21, therefore. is classified as an oncomiR (Ribas & Lupold, 2010) and proposed to be a potential non-invasive diagnostic marker for DLBCL and possibly other cancers (Lawrie *et al.*, 2008). Our analysis also showed that miR-21 is elevated in PGI-DLBCL patients as compared with healthy controls. Moreover, high miR-21 expression is associated with low OS and PFS. This is consistent with early results that circulating miR-21 level in DLBCL patients is higher than those in serum of control healthy cases (Chen *et al.*, 2014). However, we did not find a correlation between the miR-21 level and tumor stage while in the early study, the miR-21 level was higher in stages I and II than in stages III and IV (Chen *et al.*, 2014). The reason for this is unclear. In addition, miR-21 expression is also elevated in natural killer-cell lymphoma/leukemia (Yamanaka *et al.*, 2009), Hodgkin lymphoma cell lines (Gibcus *et al.*, 2009) and primary central nervous system lymphoma (Baraniskin *et al.*, 2012). All these findings suggest that miR-21 is an oncogene that promotes the occurrence and development of lymphoma. Mice overexpressing miR-21 leads to a pre-B malignant lymphoid-like phenotype with increased size and weight of hematopoietic organs such as spleen, bone

Table 3. Correlation analysis of miR-21 expression level with clinicopathological characteristics in PGI-DLBCL patients

Variables	High miR-21		Low miR-21		*P* values
	N %		N %		
Age, n (%)					0.114
<60	34	38.6	30	44.1	
≥60	54	61.4	38	55.9	
Gender, n (%)					0.214
Male	55	62.5	33	48.5	
Female	33	37.5	35	51.5	
Ann Arbor stages, n (%)					0.021
I–II	22	25.0	25	36.8	
III–IV	66	75.0	43	63.2	
IPI, n (%)					0.012
0–1	22	25.0	34	50.0	
2–3	32	36.4	13	19.1	
4–5	34	38.6	21	30.9	
Lactate dehydrogenase, n (%)					0.324
Normal	42	47.7	30	44.1	
High	46	52.3	38	55.9	
Ki-67, n (%)					0.001
<70%	39	44.3	27	39.7	
≥70 %	49	55.7	41	60.3	
Bcl2, n (%)					0.012
<50%	8	9.1	14	20.6	
≥50%	80	90.9	54	79.4	
Bcl6, n (%)					0.022
<30%	13	24.1	21	30.9	
≥30 %	75	85.2	47	69.1	
C-MYC, n (%)					0.015
<30%	11	12.5	29	42.6	
≥30%	77	87.5	39	57.4	
Cell of origin, n (%)					0.115
GC	77	87.5	46	67.6	
Non-GC	11	12.5	22	32.4	
Serum CRP, n (%)					0.312
Normal	45	51.1	23	33.8	
High	43	48.9	45	66.2	

HR, hazard ratio; CI, confidence intervals; IPI, International Prognostic Index; GC, germinal-center; non-GC, non germinal center; CRP, C-reactive protein

marrow and thymus and when miR-21 was inactivated, the tumors regressed completely in a few days. These results demonstrate that miR-21 may be targeted for human cancer treatment, such as through inactivation (Medina *et al*., 2010) since the main target gene of miR-21 is the classical tumor suppressor gene phosphatase and tensin homolog (PTEN) that suppresses the P13K/Akt signaling pathways and promotes the proliferation of tumor cells (Rai *et al*., 2008).

In addition, studies have shown that miR-21 also acts as an oncogene in other tumors. It is highly expressed in breast tumors. Once inhibited with anti-miR-21 oligonucleotides, increased apoptosis and decreased cell growth are induced in breast cancer cells and cell proliferation is suppressed, partially due to downregulation of the antiapoptotic Bcl-2 in anti-miR-21-treated tumor cells (Si *et al*., 2007). In colon cancer, miR-21 is found to be an independent prognostic indicator and the high expression

Table 4. Logistic regression analysis of miR-21 expression level

Variables	Wald	OR	95% *CI*	*P*
Ann Arbor stages	1.513	1.732	0.734–2.413	0.067
IPI	1.148	0.959	0.469–1.531	0.176
Bcl-2	2.555	2.413	0.996–4.074	0.017
Bcl-6	2.426	2.883	1.192–4.443	0.015
c-MYC	3.689	0.822	0.294–1.658	0.078
Ki-67	4.940	2.453	1.938–3.231	0.011

OR, odds ratio; CI, confidence intervals; IPI, International Prognostic Index; GC, germinal-center; non-GC, non germinal center; CRP, C-reactive protein

indicates poor survival and poor therapeutic outcome (Schetter *et al.*, 2008). In pancreatic cancers, although miR-21 expression does not correlate with tumor size, differentiation, nodal status, or T stage, strong miR-21 expression is predictive of poorer outcomes compared to absent or faint/focal miR-21 expression in patients (Dillhoff *et al.*, 2008).

The molecular pathogenesis of PGI-DLBCL is similar to that of primary lymph nodes DLBCL and most DLBCLs derive from germinal center B-cells. The initiation and maintenance of the GC are dependent on BCL-6, a transcriptional repressor belonging to the BTB/POZ/Zinc Finger family of transcription factors. It is activated via translocation activation to suppress transcription, affecting the expression of genes involved in the activation and differentiation of B cells, as well as genes related to cell cycle and apoptosis, resulting in a rapid proliferative but undifferentiated state and subsequently DLBCL (Schneider *et al.*, 2011). Another gene prone to translocation is Bcl-2. In the normal B cell differentiation, this gene is not expressed in GC, but expressed once it is translocated with the J subexons of the immunoglobulin heavy chain (IgH), leading to increased anti-apoptotic ability and tumorigenesis (Nambiar *et al.*, 2011). Our analysis showed that Bcl-2 and Bcl-6 expressions are related to miR-21 level in these patients. Therefore, it is worthy to investigate how these genes interact to modulate the progress of DLBCL. Previously, miR-211 was found to target Bcl-2 to regulate autophagy and apoptosis in cervical cancer (Liu *et al.*, 2020) and Bcl-6 could be downregulated by the miR-30 family in B-lymphocytes and lymphoma cells (Lin *et al.*, 2011), suggesting that the correlation between miR-21 and Bcl-2 and Bcl-6 may result from interactions between these molecules, although the mechanisms are still unclear.

There are limitations to our study. As a single center, observational study, the sample size is relatively small and the follow-up time is not long enough to generate long term outcome results. Therefore, prospective, large-scale, multicenter studies are necessary to confirm our results and the mechanisms of interaction between this miRNA and proteins in DLBCL warrants investigations.

CONCLUSIONS

Our data demonstrate that serum miR-21 level is significantly elevated in the PGI-DLBCL patients, a high expression level of serum miR-21 is associated with worse PFS and OS. miR-21 is an independent risk factor for PFS and OS, and is also closely related to the expression of Bcl-2, Bcl-6, and Ki-67. Therefore, serum miR-21 may be used as a biomarker to monitor and manage the treatment progress and prognosis of PGI-DLBCL.

Declarations

Ethics approval and consent to participate: the Ethics Committee of Zunyi Medical University and written informed consent were obtained from every participant.

Consent for publication: N/A.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests: None.

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