Significance of the microRNA-17-92 gene cluster expressed in B-cell non-Hodgkin's lymphoma

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Abstract. To evaluate the prognostic value of the microRNA (miR)-17-92 gene cluster, the expression of miR-17-92 in B-cell non-Hodgkin's lymphoma (B-NHL) was examined. Patients with B-NHL, who received therapy in the Department of Hematology, Harbin Medical University Cancer Hospital between January 2012 and October 2014, were enrolled in the study. The expression of the miR-17-92 cluster in tumor tissue samples was detected by reverse transcription-quantitative polymerase chain reaction analysis. The overall survival (OS) and event-free survival (EFS) times were also investigated by the Kaplan-Meier method and comparisons between groups were estimated using a log-rank test. Three types of lymphoid cancer cells with wild-type (WT), knockout of miR-17-92 (KO), and overexpression of miR-17-92 (TG), were utilized to establish a tumor xenograft model, and a reactive hyperplasia lymph cell was used as a control. The tumor incubation times and weights were examined. A total of 71 patients with B-NHL were registered. No significant correlations were identified between the expression of miR-17-92 and clinical factors (P>0.05). Members of the miR-17-92 cluster exhibited various expression in the subtypes of B-NHL, and the difference between follicular lymphoma (FL) and germinal center B-cell like (GBC) was most marked. The overexpression of miR-18, miR-19a, and miR-92a induced a marked reduction in the OS of patients with B-NHL, and high-levels of miR-19a and miR-92a led to a decline in EFS. The overexpression of miR-17-92 shortened the duration of incubation required for visualization of the xenograft tumor, whereas knockout led to inhibition of tumor formation. The expression of miR-17-92 in FL differed significantly from that in GBC, and miR-19a may have a crucial effect on the OS and EFS of patients with B-NHL.

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Introduction

Non-Hodgkin's lymphoma (NHL), a heterogeneous group of lymphoid-derived malignancies, is the seventh most prevalent type of cancer. According to the incidence trend, ~74,680 newly diagnosed cases and 19,910 cases of mortality were estimated to occur in 2016 in the USA (1). According to its origination, NHL can be divided into three types: B-cell origin, T-cell origin, and natural killer (NK)-cell origin (2). Among cases, ~80-90% are B-cell origin, namely B-NHL (3). Specifically, diffuse B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), follicular lymphoma (FL), chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), and Burkitt lymphoma (BL) are the most prevalent types of B-NHL (2). Although there have been multiple trials in the treatment of B-NHL, due to the diverse clinical and pathological performances, the diagnosis and prognosis of B-NHL and its underlying mechanism remain to be fully elucidated (4).

MicroRNAs (miRNAs) are a type of short, non-coding RNA molecule (18-22 nt) which are mainly known to be involved in the regulation of gene expression by binding to the 3'-untranslated regions (3'-UTR) of target mRNAs (4). In previous years, miRNAs have been found to be important in several types of cancer, including stomach (5), colon (6) and ovarian cancer (7), in addition to B-NHL (8). Previous studies have reported that aberrant high levels of miRNA (miR)-221, miR-21 and miR-155 are significant indicators of a poor prognosis of DLBCL (9-11). Akao *et al* (12) documented that the expression levels of miR-143 and miR-145 were downregulated in B-cell malignancies, and that increasing the expression of both induced dose-dependent growth-inhibition in BL cell lines. miR-150 and miR-16 have also been observed to have elevated expression in CLL (13,14). Taken together, this evidence indicates that miRNAs may be closely correlated with the development of B-NHL.

The miR-17-92 gene cluster is a commonly gene amplified loci in the non-protein-coding gene C13orf25 at 13q31.3 in several types of solid tumor and lymphoproliferative disorders (15,16). In this region, six tandem loop hairpin structures are contained and six mature miRNAs, including miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a, are ultimately produced and involved in the regulation of cellular processes (15). The miR-17-92 cluster is also crucial in the development of B-NHL. Tagawa *et al* (17) documented that c-Myc can not only promote the transcription of miR-17-92

cluster, but can also act as a target of the miR-17-92 cluster. A high-level of miR-17-92 cluster expression also results in the poor survival rate of patients with MCL (18). With the exception of MCL, the miR-17-92 gene cluster is also upregulated in DLBCL and ALL, and correlated with a poorer prognosis (19-21). Together, the above evidence indicates that miR-17-92 may be crucial in B-NHL, however, the detailed expression of mature miRNA in different types of B-NHL remains to be elucidated. Therefore, the present study aimed to examine the specific expression of the miR-17-92 gene cluster in different types of B-NHL, in order to provide novel insights into the treatment and prognostic prediction of B-NHL.

Materials and methods

Patients and enrolment criteria. Between January 2012 and October 2014, 71 patients, who were first diagnosed with B-NHL in the Department of Hematology, Harbin Medical University Cancer Hospital (Harbin, China), were enrolled in the present study. The basic information of patients, including gender, age, B symptoms, clinical stage, international prognostic index (IPI) score and the level of lactic dehydrogenase, were also collected. Patients were enrolled if they met the following inclusive criteria: i) Patients were preliminary diagnosed with B-NHL; ii) the diagnostic result was confirmed by two pathologists; iii) the content of tumor cells was >80%. In addition, patients were excluded if they had other types of tumor, if the diagnostic results had no definite pathological significance, or if certain types of tumor were not confirmed. Additionally, five patients with reactive hyperplasia lymph nodes were enrolled as controls. The follow-up was ended by recurrence or the occurrence of patient mortality and the final follow-up time was June 30th, 2016. The overall survival (OS) was defined as the time from diagnosis to patient mortality from any cause, and event-free survival (EFS) was designed as the time from diagnosis to disease progression or mortality from any cause, whichever occurred first. All patients were informed and provided signed consent, and the clinical investigation was authorized by the Ethics Committee of Harbin Medical University Cancer Hospital.

Cells and culture. Primary mouse lymphoma cells of wild-type (WT), which had normal expression of the miR-17-92 gene cluster; knockout (KO), which had deficient expression of the miR-17-92 gene cluster; and TG, which had 3-4 times higher expression of the miR-17-92 gene cluster than normal, were provided by the Ron Levy Laboratory of Stanford University (Stanford, CA, USA) (22). All cell lines were maintained in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in a humidified incubator with a 5% CO_2 atmosphere.

RNA extraction. Following collection of the tumor and inflammatory tissues, signal cell suspension was produced. According to the manufacturer's protocol, total RNA of the cells was extracted using an RNA extraction kit (Omega Bio-Tek, Inc., Norcross, GA, USA). In addition, the concentration and purity of the RNA were measured using a NanoDrop Nd-1000 spectrophotometer (Thermo Fisher Scientific, Inc.).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Based on the concentration of RNA, the RNA in each sample was adjusted to the same concentration and a reverse transcription kit (TaqMan® MicroRNA assay; Applied Biosystems; Thermo Fisher Scientific, Inc.) was utilized to synthesize cDNA according to the manufacturer's protocol. The RT primers were designed as follows: miR-17, 5'-GTCGTATCCAGTGCG TGTCGTGGAGTCGGCAATTGCACTGGATACGACCTA CCTG-3'; miR-18a, 5'-GTCGTATCCAGTGCGTGTCGT GGAGTCGGCAATTGCACTGGATACGACCTATCTG-3'; miR-20a, 5'-GTCGTATCCAGTGCGTGTCGTGGAGTC GGCAATTGCACTGGATACGACCTACCTG-3'; and U6, 5'-CGCTTCACGAATTTGCGTATCAT-3'. Subsequently, the synthesized cDNA was used as template to detect the level of the miR-17-92 gene cluster in the B-NHL and control groups via RT-qPCR analysis (Quantifier Human DNA Quantification kit, Thermo Fisher Scientific, Inc.) using the following conditions: 95˚C for 10 min, and 40 cycles of 95˚C for 10 min, 95˚C for 15 sec, and 60˚C for 31 sec. The total volume of the reaction system was 20 μl, including 1.33 μl cDNA, 10 μl 2X TaqMan® GeneExpression Master Mix, 1 μl 20X Real time Primer and 7.67 μl dH_2O. U6 was set as internal control of this procedure. The primers were synthesized as follows: miR-17, sense 5'-ATCCAGTGCGTGTCG TG-3' and antisense 5'-TGCTTAAAGTGCTTACAGTG-3'; miR-18a, sense 5'-ATCCAGTGCGTGTCGTG-3' and antisense 5'-TGCTTAAGGTGCATCTAGTG-3'; miR20, sense 5'-ATCCAGTGCGTGTCGTG-3' and antisense 5'-TGCTTA AAGTGCTTAATAGTG-3'; miR-17-92, sense 5'-CTGTCG CCCAATCAAACTG-3' and antisense 5'-GTCACAATC CCCACCAAAC-3'; and U6, sense 5'-GCTTCGGCAGCA CATATACTAAAAT-3' and antisense 5'-CGCTTCACGAAT TTGCGTATCAT-3' (Beijing Aoke Biotechnology, Beijing, China). The relative expression of the miRNAs was calculated by the $2^{-\Delta\Delta Cq}$ method (23).

Tumor formation in nude mice. When sufficient numbers of the four types of cells, including WT, KO and TG lymphoma cells obtained from mice and reactive hyperplasia lymph cells obtained from mouse lymph nodes, were obtained. A total of 18 female Balb/c nude mice (3-4 weeks old; weighing 16-20 g) in SPC conditions purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd were used to perform a tumor formation assay. The mice were housed at 22±2˚C with a 12-h light/dark cycle and had free access to regular diet and purified water. Saline was used to adjust the cell concentration. With the same concentration, $4x10^5$ cells of each type were injected into the subcutaneous tissue of the shoulders. In the same mouse, the left shoulder was injected with cells and the right shoulder was injected with the same volume of saline. There were six mice for each cell type. Following inoculation the lengths and diameters of the tumors were measured persistently, and the volumes of the tumors were calculated using the following formula: Volume=(π/6) x (length x diameter x diameter). After 6 weeks, the mice were sacrificed by cervical dislocation and the weights of the tumors were estimated.

Statistical analysis. In the present study, SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA) was utilized to conduct

statistical analyses. The correlations between the expression of the miRNA-17-92 gene cluster and different clinical factors were estimated using χ^2 test. Continuous comparisons among groups were estimated by two-way analysis of variance followed Fisher's LSD test. Survival analyses of the mice were evaluated using the Kaplan-Meier method, and comparisons between two groups were assessed using the log-rank test. P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical information and expression of the miR-17-92 gene cluster in patients. A total of 71 cases of B-NHL were confirmed with assured pathological significance, and were enrolled in the present study, including 48 cases of DLBCL (ABC, 33 cases; GBC, 15 cases), five cases of MCL, two cases of BL, eight cases of CLL/SLL, and eight cases of FL. The detailed clinical information of patients is listed in Table I. Based on the χ^2 test, no significant correlations were identified between the expression of the miR-17-92 gene cluster and gender, age, lactate dehydrogenase (LDH), presence of B symptoms, clinical stage or IPI score (Table II).

To obtain further insight into the development of lymphoma, patients with reactive lymphoid hyperplasia were collected as a control and six mature miRNAs (miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a) in the miR-17-92 cluster of the control and lymphoma patients were evaluated with U6 as the internal control. Compared with the control group, 19/71 (26.76%) patients with lymphoma were identified with overexpression of the miR-17-92 gene cluster (Fig. 1). Following detailed analysis, 25% of the DLBCL cases had significant upregulation of the miR-17-92 gene cluster, including miR-17, miR-18, miR-19b and miR-92, compared with the control (P<0.05). Significant upregulation of the miR-17-92 gene cluster was also identified in two cases of BL and two cases of MCL, and this upregulation was observed for all six members of the gene cluster compared with the control (P<0.05). In addition, the overexpression of miR-17-92 was observed in two cases of FL, and marked increases in the levels of miR-18 and miR-20a were observed (P<0.05). Only one case of CLL/SLL showed enhanced expression of the miR-17-92 cluster, with specific upregulation identified in miR-19a, miR-19b and miR-92, compared with the control (P<0.05).

Expression of the miR-17-92 gene cluster in different types of B-NHL. Based on the χ^2 test, comparisons of the miR-17-92 gene cluster between different types of B-NHL were made (Table III). Compared with ABC, significantly lower expression levels of miR-17-92 gene cluster members, with the exception of miR-18, were identified in patients with GCB (P<0.05), however, no significant difference was detected in patients with FL (P>0.05). Compared with GCB, significantly higher expression was observed in all members of the miR-17-92 gene cluster in patients with FL (P<0.05), however, these differences disappeared when specifically compared with stage III FL, with the exception of miR-20a (P>0.05). Overexpressed levels of miR-17, miR-18b, miR-19b, miR-20a and miR-92a were also identified in 80% of patients with DLBCL compared

Table I. Clinical information of the 71 enrolled patients with B-NHL.

	DLBCL					
Factor	ABC	GCB	MCL	BL	CLL/SLL	FL
Gender						
Male	20	10	3	1	6	5
Female	13	5	2	1	2	3
Age (years)						
≥60	22	9	4	1	5	4
<60	11	6	1	1	3	4
LDH						
Increased	28	8	4	1	2	1
Normal	5	7	1	1	6	7
B symptoms						
Yes	26	10	4	2	4	1
No	7	5	1	0	4	7
Clinical stage						
I+II	12	9	3	0	4	2
III+IV	21	6	2	2	4	6
IPI score						
High-risk + median high-risk	19	8		2	5	2
Low-risk + median low-risk	14	7	2	0	3	6

DLBCL, diffuse large B cell lymphoma; ABC, activated B-cell like; GBC, germinal center B-cell like; MCL, mantle cell lymphoma; BL, Burkitt lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FL, follicular lymphoma; B-NHL, B-cell non-Hodgkin's lymphoma; LDH, lactate dehydrogenase; IPI, International Prognostic Index.

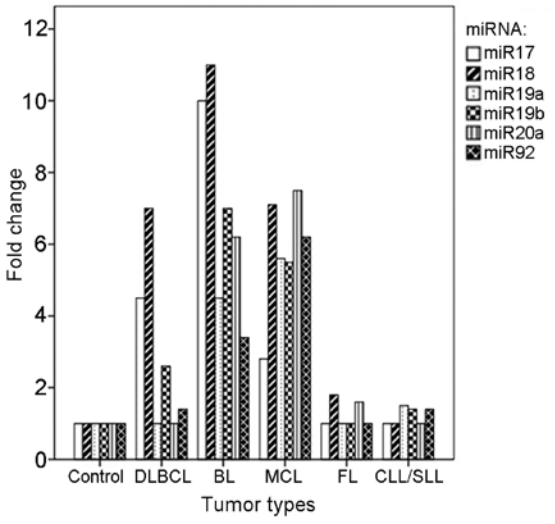

Figure 1. Expression levels of miR-17, miR-18, miR19a, miR-19b, miR-20a and miR-92 in different subtypes of B-NHL. miR, microRNA; DLBCL, diffuse large B cell lymphoma; ABC, activated B-cell like; GBC, germinal center B-cell like; MCL, mantle cell lymphoma; BL, Burkitt lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FL, follicular lymphoma; B-NHL, B-cell non-Hodgkin's lymphoma.

Table II. Correlations between microRNA-17-92 gene cluster and clinical information.

Factor	Total	Downregulated	Upregulated	χ^2	P-value
Gender					
Male	49	20	29	0.134	0.714
Female	22	10	12		
Age (years)					
≥60	48	22	26	0.250	0.617
<60	32	12	11		
LDH					
Increased	46	25	21	1.334	0.248
Normal	25	10	15		
B symptoms					
Yes	48	20	28	0.021	0.885
No	23	10	13		
Clinical stage					
I+II	32	10	22	2.891	0.089
III+IV	39	20	19		
IPI score					
High-risk + median high-risk	41	20	21	0.031	0.860
Low-risk + median low-risk	30	14	16		

LDH, lactate dehydrogenase; IPI, International Prognostic Index.

Table III. Comparisons of the miR-17-92 gene cluster among different types of B-NHL.

Type	miR-17 (FC)	miR-18 (FC)	miR-19a (FC)	miR-19b (FC)	miR-20a (FC)	miR-92 (FC)	Increase (%)
ABC vs. GCB	S	NS	S	S	S	S	80
FL vs. ABC	NS		NS	NS	NS	NS	70
FL vs. GCB	S	S	S	S	S	S	100
GCB vs. FL III		NS		NS	S	NS	100
DLBCL vs. nt-FL	S	NS		NS	S	NS	80
DLBCL-nt vs. DLBCL-t		S		S		S	85
MCL vs. CLL/SLL					NS		90

DLBCL, diffuse large B cell lymphoma; ABC, activated B-cell like; GBC, germinal center B-cell like; MCL, mantle cell lymphoma; BL, Burkitt lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; FL, follicular lymphoma; B-NHL, B-cell non-Hodgkin's lymphoma; nt, non-transformed; t, transformed; FC, fold-change; S, significant (P<0.05); NS, not significant (P>0.05).

with non-transformed FL, although differences were only significant for the expression of miR-17 and miR-20a (P<0.05). Patients with DLBCL derived from non-transformed FL also had higher expression levels of miR-18, miR-19b and miR-92a, compared with patients with DLBCL derived from transformed FL (P<0.05). No significant difference was identified between MCL and CLL/SLL (P>0.05).

Influence of overexpression of the miR-17-92 gene cluster on the prognosis of patients with B-NHL.

Influence of overexpression of the miR-17-92 gene cluster on OS. Compared with the average expression levels of miRNAs in the reactive hyperplasia lymph node group, changes in the expression of miRNAs in B-NHL were determined, and the OS of patients was analyzed. According to the analytical results, the OS of patients with overexpressed miR-18 (Fig. 2A), miR-19a (Fig. 2B) and miR-20a (Fig. 2C) were shortened compared with that of patients with normal expression levels (Table IV).

Influence of overexpression of the miR-17-92 gene cluster on EFS. Similar to the analysis of OS, the influence of overexpression of the miR-17-92 gene cluster on ESF was investigated. Following analysis, marked reductions EFS

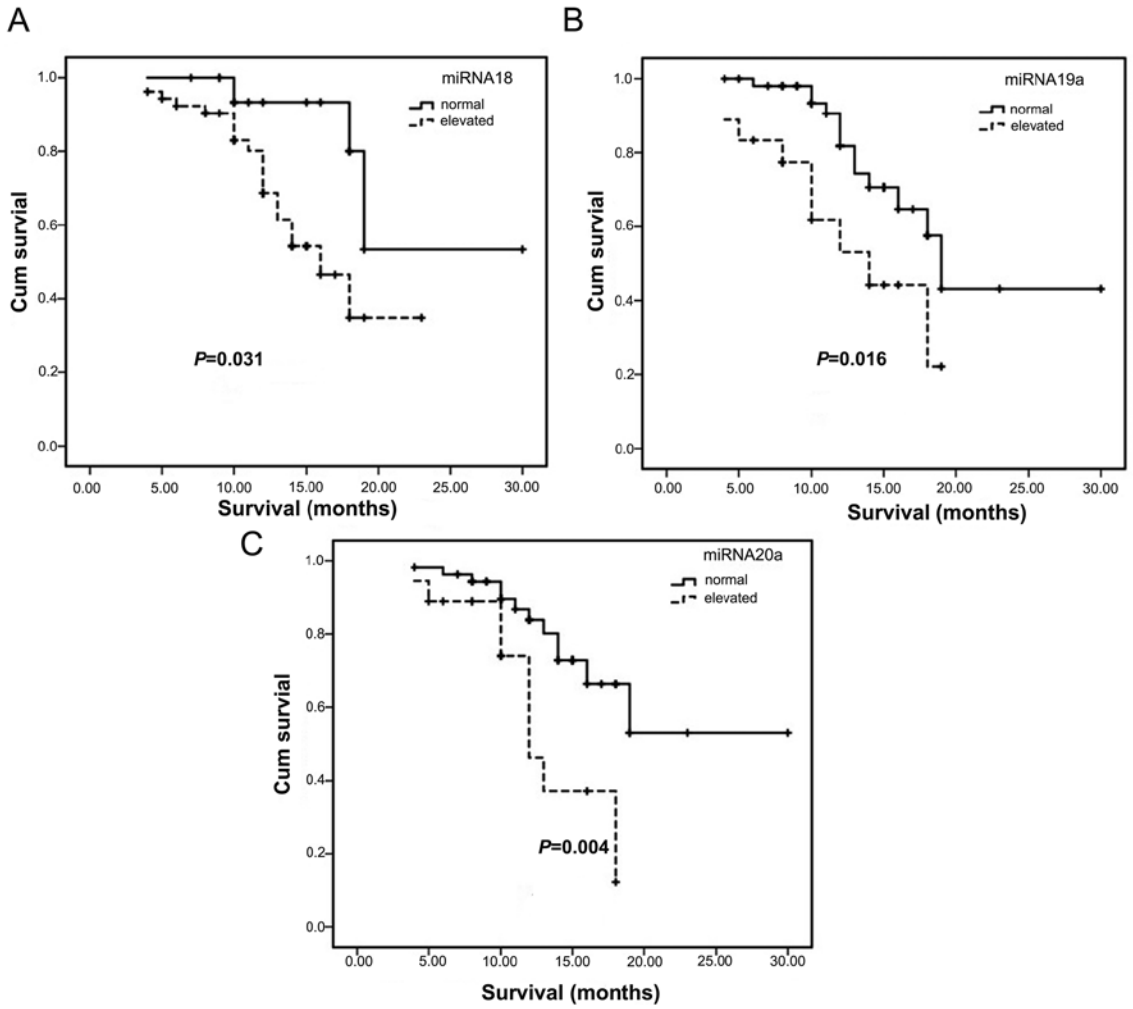

Figure 2. Overall survival analyses of patients with B-NHL. Results in patients with normal with increased expression of (A) miR-18, (B) miR-19a and (C) miR-20a. miR/miRNA, microRNA.

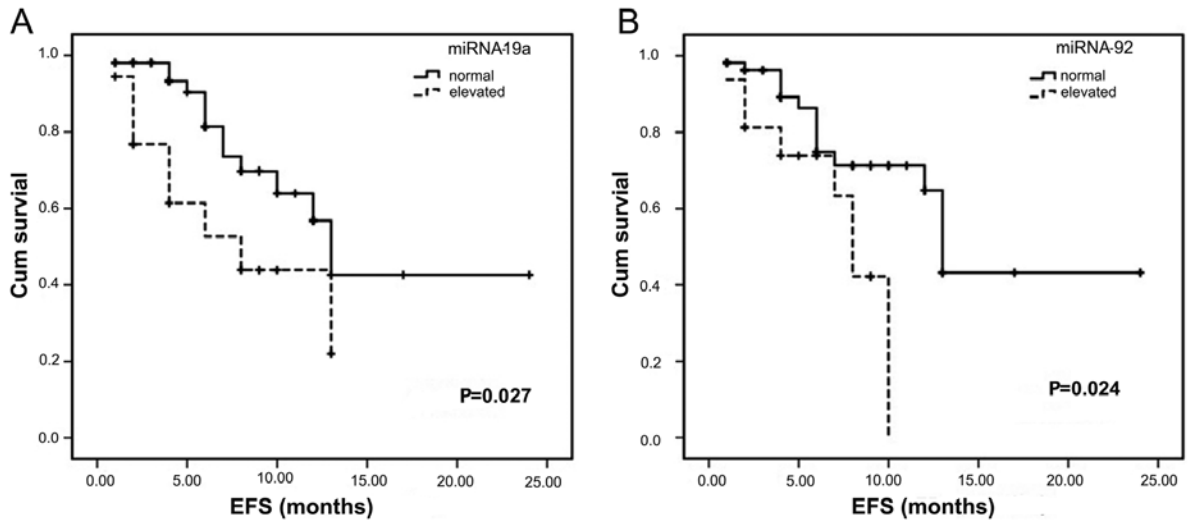

Figure 3. EFS analyses of patients with B-NHL. Results in patients with normal and increased expression of (A) miR-19a and (B) miR-92a. EFS, event-free survival; miR/miRNA, microRNA.

were identified in patients with overexpressed miR-19a and miR-92a (Fig. 3A and B) compared with the patients with normal levels (Table V).

Tumor formation assay in nude mice. According to the results described above, the miR-17-92 gene cluster may have a potential effect in promoting the development of B-NHL. In order to

Table IV. Median OS of patients with differing expression of miRNAs.

miRNA	Variation	Patients (n)	OS (months)	P-value
miRNA-17	Increase	16	18	0.057
	Normal	55	19	
miRNA-18	Increase	18	16	0.031
	Normal	53	20	
miRNA-19a	Increase	18	14	0.016
	Normal	53	19	
miRNA-19b	Increase	16	18	0.321
	Normal	55	19	
miRNA-20a	Increase	18	12	0.004
	Normal	53	25	
miRNA-92a	Increase	16	18	0.087
	Normal	55	19	

miRNA, microRNA; OS, overall survival.

Table V. Median EFS of patients with differing expression of miRNAs.

miRNA	Variation	Patients (n)	EFS (months)	P-value
miRNA-17	Increase	16	10	0.052
	Normal	55	13	
miRNA-18	Increase	18	12	0.058
	Normal	53	13	
miRNA-19a	Increase	18	8	0.027
	Normal	53	13	
miRNA-19b	Increase	16	13	0.407
	Normal	55	13	
miRNA-20a	Increase	18	10	0.106
	Normal	53	13	
miRNA-92a	Increase	16	8	0.024
	Normal	55	13	

miRNA, microRNA; EFS, event-free survival.

evaluate the biofunctions of the miR-17-92 gene cluster *in vivo*, a tumor formation assay in nude mice was performed. A total of 24 samples in four groups (WT, KO, TG and reactive hyperplasia lymph node) were used for this assay, and all animals in the WT and TG groups exhibited tumor formation as a result, with the exception of two cases in the TG group, in which the mice died 10 days following injection (Fig. 4). However, no tumor nodes were produced in the KO or reactive hyperplasia lymph node groups following injection with the same concentration of KO and reactive hyperplasia lymph node cells. In terms of the formation of tumors, the duration of incubation required until tumor visualization was significantly shorter in the TG group than in the WT group ($P<0.05$; Fig. 5A). The

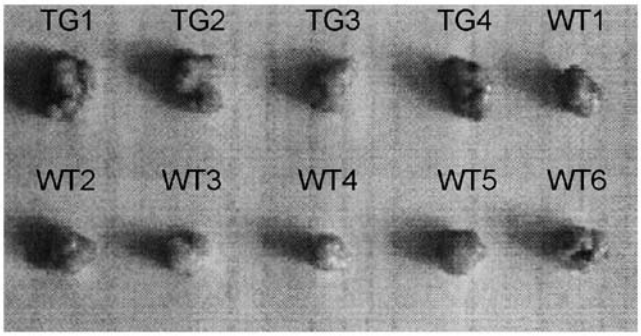

Figure 4. Tumor formation of xenograft tumors in nude mice. WT, wild-type lymphoma cell with normal expression of miR-17-92 gene cluster; TG, lymphoma cell with 3-4-fold higher expression of the miR-17-92 gene cluster. miR, microRNA.

tumor weights and volumes in the TG group were also higher than those in the WT group, although no significant difference was identified ($P>0.05$; Fig. 5B and C). These results indicate that the miR-17-92 gene cluster may have a potential carcinogenic characteristic, and this feature may correlate with the occurrence and development of lymphoma.

Discussion

In the present study, the six mature miRNAs in the miR-17-92 gene cluster were detected in several types of B-NHL. The results showed that ~27% of B-NHL cases presented with a high level of the miR-17-92 gene cluster, including DLBCL, BL, MCL, FL and CLL/SLL, although the levels of the six mature miRNAs derived from miR-17-92, (miR-17, miR-18, miR-19a, miR-19b, miR-20a and miR-92) differed between these types. The investigation of OS and EFS demonstrated that overexpressed miRNA18, miR-19a and miR-20a have a positive effect on the OS of patients with B-NHL, and elevated expression levels of miR-19a and miR-92a led to a poor EFS for patients with B-NHL. In addition, the tumor formation assay indicated that overexpression of the miR-17-92 gene cluster led to acceleration in the occurrence of tumors in nude mice, however, the tumor weights did not differ.

miR-17-92 is encoded by C13orf25, which is a unique non-coding RNA in the aberrant amplification of 13q31-32 in B-NHL. Therefore, it is useful to investigate the mechanism of B-NHL via examining the associated biofunction of the miR-17-92 gene cluster (15). Among previously published literature, c-Myc is the most referred to molecule, which is key in cell apoptosis (15). A mouse model indicates that c-Myc can activate the expression of the miR-17-92 gene cluster, and can also be regulated by the miR-17-92 gene cluster. This indicates that there may be negative regulation between c-Myc and the miR-17-92 gene cluster, and loss of control of this regulation is important for the development of B-NHL (17). miR-17-92 also can inhibit the expression levels of phosphatase and tensin homolog (PTEN), P21, and Bcl-2-like 11 (Bim) to promote proliferation and suppress the apoptosis of cancer cells (24). The xenograft tumor assay confirmed that the incubation duration of mice with the overexpressed miR-17-92 gene cluster was shorter than that with normal levels, however, the deletion of miR-17-92 resulted in failure of tumor formation.

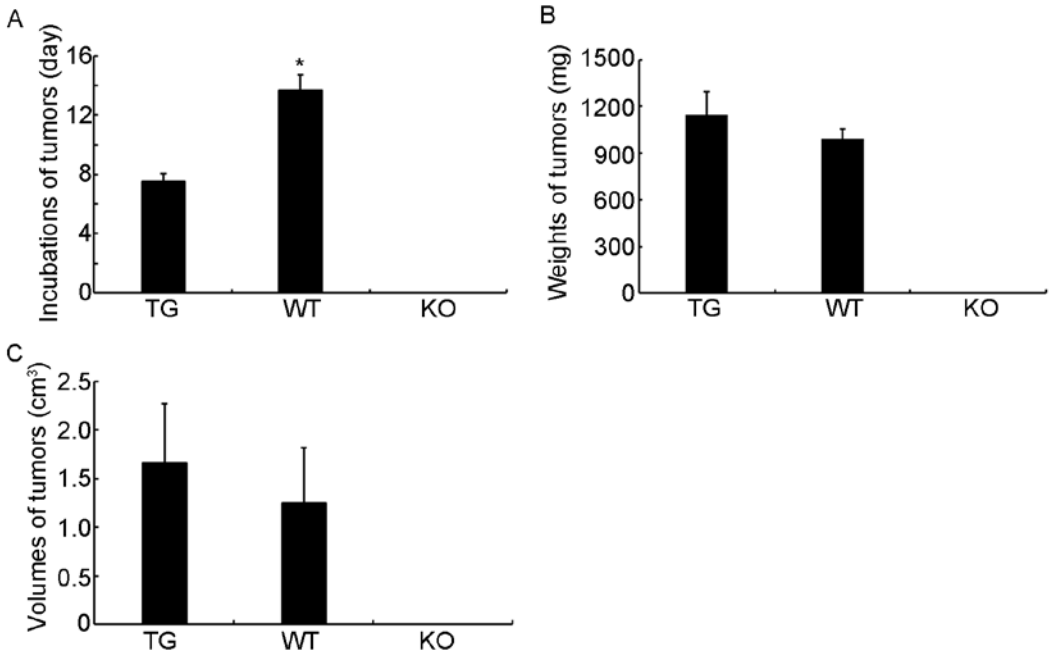

Figure 5. Tumor incubation duration, weights and volumes detected in TG, WT and KO groups. (A) Tumor incubation time for first appearance visualized by the naked eye. *P<0.05 vs. WT group. (B) Weights of tumors in different groups following incubation for 6 weeks. (C) Volumes of tumors in different group following incubation for 6 weeks. TG, lymphoma cell with 3-4-fold higher expression of the miR-17-92 gene cluster; WT, wild-type lymphoma cell with normal expression of the miR-17-92 gene cluster; KO, lymphoma cell with knockout of the miR-17-92 gene cluster; miR, microRNA.

This indicates that miR-17-92 may serve as an oncogene in the occurrence and development of B-NHL.

DLBCL is the most common type of B-NHL and accounts for ~33% (15). A previous study showed that >18% of patients with DLBCL had 2-36-fold higher expression of the miR-17-92 gene cluster (25). However, due to the high heterogeneity of DLBCL, the levels of members of the miR-17-92 cluster are not consistent in ABC and GCB. Robertus *et al* (26) confirmed that the expression of miR-19b is only elevated in ABC. In the present study, >27% of DLBCL cases had elevated expression of the miR-17-92 gene cluster. With the exception of miR-18, the levels of the remaining miRNAs in the miR-17-92 gene cluster were significantly higher than those in the GCB subtype. Significant differences were also identified between transformed and untransformed DLBCL in the expression levels of miR-18, miR-19b and miR-92. Controversially, Lenz *et al* (27) reported that 13q31 amplification frequently occurs in GCB but not ABC, and that the overexpression of miR-17-92 is always correlated with high levels of MYC and its target genes. The above evidence indicates that expression of the miR-17-92 gene cluster is not coincident in different DLBCL subtypes, however, the particular expression levels in these subtypes require further elucidation.

FL is the most frequent indolent tumor type of B-NHL, and 90% of this type can transform into DLBCL 31. Zhao *et al* (28) reported that the increased expression of miR-17 can act as an important marker in the invasive transformation and prognosis of FL. Studies by Lawrie *et al* (29) and Fassina *et al* (30) also suggested that miR-17-92 profiling can be utilized to distinguish morphologically similar disease between grade III of FL, and *de novo* and transformed DLBCL. In the present study, a marked difference in expression of the miR-17-92 gene cluster was also observed between FL and GCB, but not ABC. This suggests that miR-17-92 may be a useful biomarker in the distinction of FL and GCB. In addition, the distinction of non-transformed FL and DLBCL was only detected in miR-17 and miR-20a, and the difference between grade III FL and GCB was only identified in miR-20a. Taken together, these findings suggest that the expression of miR-17-92 may serve as a biomarker to distinguish FL and GCB, although the differences in the different states of FL and the subtype of DLBCL remain to be fully elucidated, of which further investigation is required.

The miR-17-92 gene cluster is not only involved in the development of B-NHL, but is also affects the prognosis of patients with B-NHL. In the present study, the estimations of OS and EFS showed that the OS rates were significantly lower in patients with overexpressed miR-18, miR-19a and miR-20a, and that the EFS rates were markedly decreased in patients with high levels of miR-19a and miR-92a. This indicates that miR-19a may be important in the OS and EFS rates of patients with B-NHL. It is documented that miR-19a and miR-19b can downregulate suppressor of cytokine signaling 1, a negative regulator of the interleukin 6 pathway, inducing constitutive activation of the Janus kinase/signal transducer and activator of transcription 3 (STAT3) signaling pathway and contributing to myelomagenesis (31). The pharmacological inhibition of STAT3 induces a dose-dependent reduction of the miR-17-92 cluster (32). This may be a potential mechanism for miR-19a in B-NHL and may serve as a biomarker in the prognosis of B-NHL. miR-19 can also negatively regulate the phosphatidylinositol-3-kinase pathway via silencing the genes PTEN, protein phosphatase 2, Bim and protein kinase AMP-activated α1 in acute leukemia (33). As a lymphoid malignancy, this mechanism may also be involved in B-NHL, although confirmation is required.

In conclusion, it was made apparent that the miR-17-92 gene cluster is important in the development and prognosis

of B-NHL. Among the subtypes of B-NHL, the expression levels of the six members of miR-17-92 varied, and the distinction between FL and GCB was most evident. Based on this evidence, it can be inferred that miR-19a may be important in the prognosis of B-NHL, although the detailed mechanism requires further elucidation. However, compared with existing evidence, controversies remain, and more detailed investigation based on a large sample is required.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

SY and CJ were responsible for the conception and design of the research, and drafting the manuscript. LQ performed the data acquisition. LZ performed the data analysis and interpretation. YT and AL participated in the design of the study and performed the statistical analysis. All authors have read and approved the manuscript.

Ethics approval and consent to participate

All patients were informed and provided signed consent, and the clinical investigation was authorized by the Ethics Committee of Harbin Medical University Cancer Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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