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Original Article

Prognostic role of serum miRNA-16 in primary gastric lymphoma

Shanshan Gu[1,2], Xiaogan Wang[1], Lixia Feng[1], Yan Xie[1], Lingyan Ping[1], Weiping Liu[1], Jiao Li[1], Yalu Liu[1], Ning Ding[1], Yuqin Song[1], Jun Zhu[1]

[1]*Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Lymphoma, Peking University Cancer Hospital and Institute, Beijing, China;* [2]*Myasthenia Gravis Treatment Center of Hebei Province, First Hospital of Shijiazhuang, Hebei, China*

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Abstract: Primary gastric lymphoma (PGL) is the most common extranodal non-Hodgkin lymphoma. However, uniform criteria for treatment and outcome evaluation are lacking. MicroRNA-16 (miRNA-16) has been shown to exhibit tumor-suppressive properties in many cancers and is a good biomarker of prognosis. We detected the expression level of miRNA-16 in 91 patients with PGL by qPCR and analyzed the prognostic significance. The proportion of patients with high expression of miRNA-16 was greater in patients with disease onset at age ≤ 60 years (P = 0.027), early-stage disease (P = 0.06), and marginal zone B-cell lymphoma (P = 0.0031). Furthermore, the proportion of patients with high expression of miRNA-16 was greater in those with a complete response than in those without a complete response (P = 0.054). However, miRNA-16 expression was not a statistically significant predictor of survival. These results suggest that miRNA-16 is a useful biomarker of disease prognosis in patients with PGL.

Keywords: Primary gastric lymphoma, miRNA-16, prognostic indicator

Introduction

Primary gastric lymphoma (PGL) is the most common extranodal non-Hodgkin lymphoma (NHL) representing nearly 5% of primary gastric neoplasms. The most common pathological type of gastrointestinal lymphoma is the diffuse large B-cell lymphoma and patients who are often diagnosed at an advanced stage (III-IV) have poor outcome. Thus, early accurate detection is urgently needed in management for PGL. Another histologic subtype of PGL is extranodal marginal zone lymphoma of mucosal-associated lymphoid tissue (MALT) [1]. MALT lymphomas are usually secondary to *Helicobacter pylori* infection, and *H. pylori* eradication therapy is an effective treatment for patients with *H pylori* (+). However, these patients still have a high risk of recurrence. Although patients receiving various treatments including surgical resection, immunotherapy, chemotherapy, radiotherapy, and *H. pylori* eradication therapy, have a 5-year overall survival of 80-95%, patients at advanced stage cannot achieve long-term survival [2]. The prognostic outcome of PGL depends on the International Prognostic Index (IPI), highly malignant transformation and mass types. However, the efficiency of IPI could be enhanced by inclusion of additional prognostic markers. Therefore, development of novel prognostic factors based on the biology of PGL is an unmet clinical need.

Mature microRNAs (miRNAs) are a family of small non-coding RNA molecules with 18-22 nucleotides that play a key role in biological processes through post-transcriptional gene regulation [3, 4]. Mature miRNAs are generated from primary miRNAs (pri-miRNAs), which are cleaved in the nucleus by the ribonuclease III protein, Drosha, to form precursor miRNAs (pre-miRNAs). Karyopherin exportin complexes transport pre-miRNAs to the cytoplasm where they are cleaved by another ribonuclease III protein, Dicer. These mature miRNAs are incorporated into the RNA-induced silencing com-

Table 1. Patient characteristics and their correlations with microRNA-16

Clinical parameter	NO.	miRNA-16		*P*	Clinical parameter	NO.	miRNA-16		*P*
		Positive	Negative				Positive	Negative	
Gender					B symptoms				
Male	46	9	37	0.136	Positive	31	8	23	0.93
Female	45	15	30		Negative	60	16	44	
Age					LDH				
> 60	32	4	28	0.027	Positive	19	6	13	0.586
≤ 60	59	20	39		Negative	71	18	53	
Stage					β2-MG				
I-II	71	22	49	0.06	Positive	13	1	12	0.122
III-IV	20	2	18		Negative	57	16	41	
HP					OB				
Positive	30	7	23	0.925	Positive	23	5	18	0.595
Negative	37	9	28		Negative	62	17	45	
IPI score					ESR				
0-2	78	22	56	0.483	Positive	37	12	25	0.639
3-5	11	2	9		Negative	26	7	19	
HCG					HbsAg				
Positive	11	2	9	0.487	Positive	4	2	2	0.266
Negative	71	20	51		Negative	84	21	63	

plex (RISC) and act as a template for RISC to recognize and bind to the 3'-untranslated region of the complementary mRNA [5-8]. Binding to the target mRNA leads to translational repression or degradation of the target message, providing a mechanism for post-transcriptional regulation of gene expression [9]. miRNAs are dysregulated in a variety of malignancies and can contribute to cancer development and progression, suggesting that miRNA expression patterns could serve as early indicators of response to personalized cancer therapies [9, 10].

Several studies recently reported that circulating miRNAs, stably detected in plasma and serum, are resistant to endogenous ribonuclease activity due to the protective activity of exosomes and Argonaute 2 [11, 12]. The expression of circulating miRNAs in patients with tumors are significantly altered from healthy people [13]. Hu *et al* described four miRNAs (miRNA-486, miRNA-30d, miRNA-1 and miR-499), which were notably associated with overall survival in non-small-cell lung cancer [14]. These miRNAs can be downregulated as a result of genomic deletion or upregulated via amplification, suggesting that they induce tumorigenesis by dysregulation of target genes, acting as either oncogenes or tumor suppressors. Importantly, analysis of circulating plasma miRNA levels in cancer patients presents a less-invasive biomarker-based screening method compared to a traditional biopsy and can facilitate diagnosis, prognosis, and choice of treatment for patients [15]. In our study, we analyzed serum from PGL patients and conducted miRNA expression profiling. As a result of our investigation of the relationships between miRNA expression levels and clinical outcomes, we identified miRNA-16 as a suitable biomarker with prognostic significance that could be used in deciding treatment courses and predicting outcomes in patients with PGL.

Materials and methods

Patients and samples

This study analyzed 91 patients at the Peking University Cancer Hospital from October 2011 to March 2016 who were diagnosed with PGL according to the standards set forth by the World Health Organization classification and PGL diagnostic criteria. Serum samples were obtained from patients before initiation of therapy. This retrospective research protocol

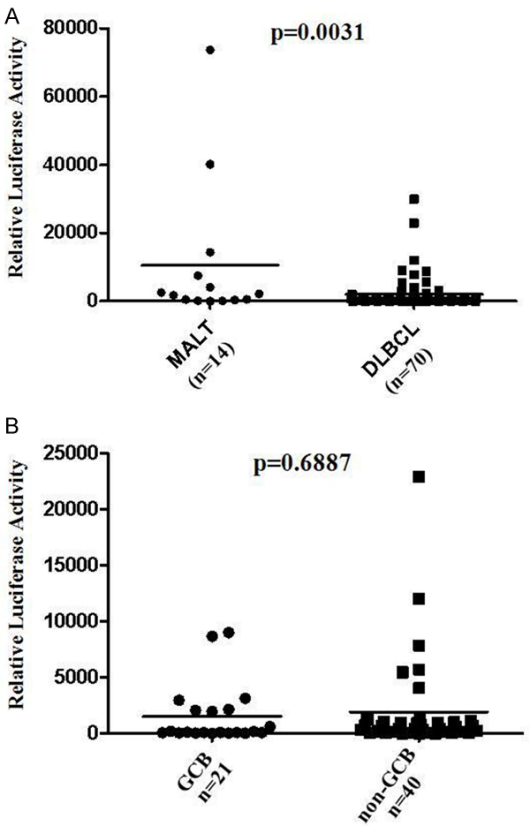

Figure 1. The expression level of microRNA-16 in PGL subgroups.

was approved by the Institutional Review Board and the Ethical Committee of Peking University School of Oncology, Beijing, China.

miRNA extraction and real-time quantitative PCR

Total RNA was extracted from serum using RNA TRIzol Extraction (Life Technologies). Real-time PCR was performed using the miDETECTA TrackTM miRNA qRT-PCR Starter Kit according to the manufacturer's instructions (RIBOBIO, Guangzhou, China).

Clinical treatments and outcomes

Clinical responses were determined by physical examination and confirmed by computed tomography or ultrasonography; the latter was only used for evaluating superficial lymph nodes. Responses were scored according to the International Working Group criteria. Overall survival (OS) was measured from day 1 of the first cycle of treatment with rituximab, cyclophosphamide, hydroxydaunomycin, oncovin/vincristine, and prednisone (R-CHOP) or R-CHOP like until the last available follow-up or death from any cause.

Statistical analysis

The Kaplan-Meier estimator was used to determine significant differences in OS. The clinical characteristics and response rates of the patients were compared using the Chi-square test. Differences between groups were considered significant at $P < 0.05$. All statistical analysis was done using SPSS software (version 19.0).

Results

Patient characteristics

Clinical characteristics of the patients in this study (45 males and 46 females) are summarized in **Table 1**. Thirty-one patients (34.0%) exhibited B symptoms, twenty patients (22.0%) were in stage 3 or 4, and 11 patients (12.1%) had intermediate-to-high or high international prognostic index (IPI) scores. The frontline treatment regimen was R-CHOP or R-CHOP-like chemotherapy (R-COP, R-COPP and R-CHOPE).

Correlation analysis between microRNA-16 and the clinical features of PGL patients

Real-time PCR analysis of miRNA-16 expression shows that patients with disease onset at age ≤ 60 years had higher expression of miRNA-16 than patients aged > 60 years at onset (P = 0.027). Patients with early stage (I-II) disease had increased expression of miRNA-16 compared with those at late stage (III-IV) disease (P = 0.06; **Table 1**).

Expression of miRNA-16 in patient subgroups

According to pathology diagnosis, all patients were classified as having DLBCL (n = 70), MALT (n = 14), or other (n = 9) forms of NHL. The DLBCL patients were sub-classified into the germinal center B cell-like (GCB) group (n = 21) and the non-GCB group (n = 40). A higher level of miRNA-16 expression in MALT lymphoma patients were evaluated compared to DLBCL patients (P = 0.0031; **Figure 1A**). No difference was observed between the GCB and non-GCB subgroups (P = 0.6887; **Figure 1B**).

Table 2. Clinical response to therapy according to microRNA-16 expression

Response	NO.	miRNA-16		*P*
		Positive	Negative	
CR	54	17	37	0.054
PR+PD+SD	26	3	23	
OR	71	18	53	0.838
PD+SD	9	2	7	

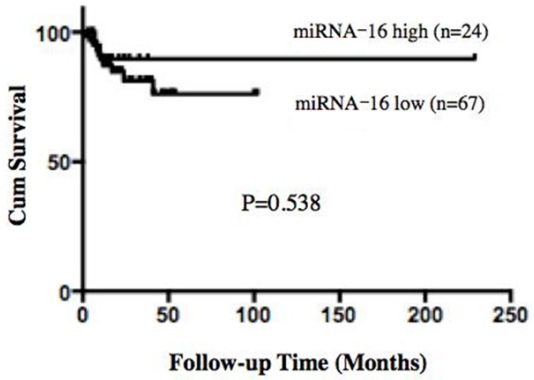

Figure 2. Kaplan-Meier curves for overall survival in PGL patients.

Impact of microRNA-16 on treatment efficacy

Of the 91 patients who were assessed for the frontline treatment regimen response to R-CHOP or R-CHOP-like chemotherapy (R-COP, R-COPP and R-CHOPE), the overall response rate (ORR) was 78% (71 of 91 patients), including a complete response (CR) rate of 59.3% (54 of 91 patients) and a partial response (PR) rate of 18.7% (17 of 91 patients). Patients with high expression of miRNA-16 exhibited higher CR rates than those with low expression of miRNA-16 (85% vs. 61.6%, $P = 0.054$; **Table 2**).

Relationship between microRNA-16 expression and overall survival

After a median follow-up time of 17 months (range, 2-229 months), seventeen (18.7%) patients relapsed or progressed, and 12 (13.2%) patients died. Therefore, a total of 91 patients were evaluated for OS. A better OR rate was observed in patients with high expression of miRNA-16 (range, 0-229) than in patients expressing low miRNA-16 levels (range, 1-102) (**Figure 2**). However, the differences between these two groups did not reach statistical significance ($P = 0.538$).

Discussion

In this retrospective analysis, differences in plasma miRNA-16 levels were found to be associated with age at onset and stage in PGL patients. Our results suggested that patients with higher expression of miRNA-16 may get more benefit from treatment. PGL is a heterogeneous malignancy, comprising different histologic subtypes and prognoses, ranging from low-grade MALT to high-grade DLBCL lymphomas. The incidence of these lymphomas has increased over the last few decades. However, PGL lacks uniform criteria for determining the best course of treatment and for the evaluation of therapeutic outcomes.

miRNAs are small (~22-nucleotides), noncoding RNAs found in higher eukaryotes and are involved in the regulation of many biological processes. Based on sequence complementarity, miRNAs bind to the mRNAs of their target genes and either inhibit their translation or accelerate their degradation [16]. Several miRNAs have been intensely studied because of their involvement in modulating the cell cycle and their dysregulation in several types of cancers. For example, a genome-wide miRNA expression analysis has been carried out in malignant lymphoma subtypes. Many of the miRNAs identified in different malignant lymphoma subsets were found to play an essential role in tumor development and progression. These miRNAs were dysregulated due to either amplification or genomic deletion, suggesting that they contributed to tumorigenesis via altered regulation of target genes, including oncogenes and tumor suppressor genes. Therefore, the study of miRNAs may provide new diagnostic tools, lead to new therapeutic strategies, and aid in the evaluation of patient prognosis.

Many genes related to the control of cell proliferation and the cell cycle are validated targets of miRNA-16 regulation [17-19]. miRNA-16 has also been demonstrated to act as a tumor suppressor in solid malignancies, such as advanced prostate cancer, where it was found to be significantly downregulated [20]. In glioma, miRNA-16 was found to suppress the migration, proliferation, and invasion of tumor cells through down-regulation of BCL2 and the NF-κB signaling pathway [21]. In breast cancer,

overexpression of miRNA-16 was demonstrated to suppress the growth and self-renewal of mouse mammary tumor stem cells and to sensitize the MCF-7 human breast cancer cell line to doxorubicin [22]. These data indicate that miRNA-16 may participate in the control of cell growth and play a tumor-suppressive role by regulating cell proliferation and apoptosis in multiple types of tumors.

In our study, the proportion of patients with high expression of miRNA-16 was greater in those with a CR to treatment than in those without a CR. Importantly, these results indicated that miRNA-16 expression was high in patients with a good prognosis. Moreover, miRNA-16 expression was higher in MALT patients than in DLBCL patients. MALT is an indolent lymphoma and has a favorable prognosis when compared with DLBCL. We also found that patients with high expression of miRNA-16 tended to have better rates of survival than those with low expression; however, these differences were not statistically significant in the survival analysis (**Figure 1**). Further investigation will be required to determine the precise mechanism by which miRNA-16 affects the pathology of PGL.

In conclusion, our results demonstrate that the miRNA-16 could serve as a predictive biomarker related to clinical outcome of PGL patients.

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Disclosure of conflict of interest

None.

Address correspondence to: Jun Zhu, Yuqin Song and Ning Ding, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Department of Lymphoma, Peking University Cancer Hospital and Institute, No. 52 Fucheng Road, Haidian District, Beijing 100142, China. Tel: +86-10-8819-6109; Fax: +86-10-8819-6115; E-mail: zhujun2017@outlook.com (JZ); songyuqin622@163.com (YQS); ningding@bjmu.edu.cn (ND)

References

[1] Psyrri A, Papageorgiou S and Economopoulos T. Primary extranodal lymphomas of stomach: clinical presentation, diagnostic pitfalls and management. Ann Oncol 2008; 19: 1992-1999.

[2] Koch P, Probst A, Berdel WE, Willich NA, Reinartz G, Brockmann J, Liersch R, del Valle F, Clasen H, Hirt C, Breitsprecher R, Schmits R, Freund M, Fietkau R, Ketterer P, Freitag EM, Hinkelbein M, Heinecke A, Parwaresch R and Tiemann M. Treatment results in localized primary gastric lymphoma: data of patients registered within the German multicenter study (GIT NHL 02/96). J Clin Oncol 2005; 23: 7050-7059.

[3] Lagos-Quintana M, Rauhut R, Lendeckel W and Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 2001; 294: 853-858.

[4] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[5] Kim VN, Han J and Siomi MC. Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 2009; 10: 126-139.

[6] Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S and Kim VN. The nuclear RNase III Drosha initiates microRNA processing. Nature 2003; 425: 415-419.

[7] Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T and Zamore PD. A cellular function for the RNA-interference enzyme dicer in the maturation of the let-7 small temporal RNA. Science 2001; 293: 834-838.

[8] Hammond SM, Boettcher S, Caudy AA, Kobayashi R and Hannon GJ. Argonaute2, a link between genetic and biochemical analyses of RNAi. Science 2001; 293: 1146-1150.

[9] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.

[10] Baer C, Claus R and Plass C. Genome-wide epigenetic regulation of miRNAs in cancer. Cancer Res 2013; 73: 473-477.

[11] Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ and Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9: 654-659.

[12] Schwarzenbach H, Nishida N, Calin GA and Pantel K. Clinical relevance of circulating cell-free microRNAs in cancer. Nat Rev Clin Oncol 2014; 11: 145-156.

[13] Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, Peterson A, Noteboom J, O'Briant KC, Allen A, Lin DW, Urban N, Drescher CW, Knudsen BS, Stirewalt DL, Gentleman R, Vessella RL, Nelson PS, Martin DB and Tewari M. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008; 105: 10513-10518.

[14] Hu Z, Chen X, Zhao Y, Tian T, Jin G, Shu Y, Chen Y, Xu L, Zen K, Zhang C and Shen H. Serum microRNA signatures identified in a genome-wide serum microRNA expression profiling predict survival of non-small-cell lung cancer. J Clin Oncol 2010; 28: 1721-1726.

[15] Wang WT and Chen YQ. Circulating miRNAs in cancer: from detection to therapy. J Hematol Oncol 2014; 7: 86.

[16] Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009; 10: 704-714.

[17] Cittelly DM, Das PM, Salvo VA, Fonseca JP, Burow ME and Jones FE. Oncogenic HER2-{Delta}16 suppresses miR-15a/16 and deregulates BCL-2 to promote endocrine resistance of breast tumors. Carcinogenesis 2010; 31: 2049-2057.

[18] Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M and Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 2005; 102: 13944-13949.

[19] Wang F, Fu XD, Zhou Y and Zhang Y. Down-regulation of the cyclin E1 oncogene expression by microRNA-16-1 induces cell cycle arrest in human cancer cells. BMB Rep 2009; 42: 725-730.

[20] Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, D'Urso L, Pagliuca A, Biffoni M, Labbaye C, Bartucci M, Muto G, Peschle C and De Maria R. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nat Med 2008; 14: 1271-1277.

[21] Yang TQ, Lu XJ, Wu TF, Ding DD, Zhao ZH, Chen GL, Xie XS, Li B, Wei YX, Guo LC, Zhang Y, Huang YL, Zhou YX and Du ZW. MicroRNA-16 inhibits glioma cell growth and invasion through suppression of BCL2 and the nuclear factor-kappaB1/MMP9 signaling pathway. Cancer Sci 2014; 105: 265-271.

[22] Zhang X, Wan G, Mlotshwa S, Vance V, Berger FG, Chen H and Lu X. Oncogenic Wip1 phosphatase is inhibited by miR-16 in the DNA damage signaling pathway. Cancer Res 2010; 70: 7176-7186.