Original Article
Prognostic role of serum miRNA-16 in primary gastric lymphoma

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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-
The role of miRNA-16 in PGL patients

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

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Table 1. Patient characteristics and their correlations with microRNA-16
The role of miRNA-16 in PGL patients

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, onco-

vin/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).

Figure 1. The expression level of microRNA-16 in PGL subgroups.
The role of miRNA-16 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 (\( P = 0.538 \)).

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

<table>
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<tr>
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<td>PD+SD</td>
<td>9</td>
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</table>

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

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.

Acknowledgements

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Disclosure of conflict of interest

None.

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References


