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Brief communication

Over expression of circulating miR-155 predicts prognosis in diffuse large B-cell lymphoma

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ABSTRACT

Introduction: The expression patterns of microRNAs in plasma are involved in potential biomarkers for several diseases. The goal of this study was to explore the expression level of miR-155 in diffuse large B-cell lymphoma (DLBCL) and its clinical significance.

Materials and methods: We used qRT-PCR to assess the peripheral blood plasma of 40 DLBCL patients for the expression of miRNA-155. The median of miR-155 expression divided the DLBCL patients into miR-155 low-expression ($miR\text{-}155^{low}$) and miR-155 high-expression ($miR\text{-}155^{high}$) groups.

Results and discussion: We found that plasma miR-155 expression was significantly up-regulated in patients with DLBCL (median expression value: 4.29, range: 1.52–27.86) compared to healthy individuals (median expression value: 2.14, range: 0.29–10.56, $P < 0.002$). Moreover, DLBCL cases with an elevated level of miR-155 had shorter overall survival (median 9 vs. 13 months, $P = 0.043$) than those with a lower miR-155 expression.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL) is a highly aggressive disease and the most frequent form of non-Hodgkin lymphoma [1]. Although most cases of DLBCL have been shown to be curable, about 35 ± 40% of cases die due to the progression of disease, while chemotherapy-related toxicity remains a problem in treated DLBCL patients [2]. Most detection and treatment choices for DLCBL cases are based on clinical features rather than quantifiable characteristics of biological parameters [3]. Until now, a general lack of precise therapy methods resulted in the current DLBCL treatment paradigms. However, the emergence of multiple molecular genetic markers as potential prognostic and molecular therapeutic targets would be important in DLBCL. The pathologic and neoplastic mechanism contributing to the abnormal biological features in DLBCL cases remains to be elucidated due to lack of precise clinical features and the incomplete response to the current therapy [4]. Although there are new developments and achievements in diagnostic and therapeutic approaches, most cases of DLBCL still have an adverse outcome. Furthermore, despite the emergence of numerous clinical biomarkers to better categorize and predict the outcome at the time of diagnosis, they are still not commonly used in clinical settings [5]. Therefore, a further investigation of specific biomarkers involved in DLBCL pathogenesis may provide a more targeted and effective treatment opportunity. MicroRNAs (miRNAs) are endogenous short (~22-nucleotide) non-protein-coding regulatory RNA molecules. Some miRNAs act as oncogenes, whereas others act as tumor suppressors. Depending on miRNAs' targets, they may provide insights into human cancer detection [6].

It is reported that specific miRNAs may be associated with the outcome in patients with DLBCL [7]. MiR-155 has been demonstrated to function in hematopoiesis and immune response, acting as an oncomiR in many malignancies, especially in B-cell lymphoproliferative disorders. In several lymphomas, the elevated expression of miR-155, including DLBCL, has been reported [8]. However, the association of higher levels of circulating miR-155 with the overall survival is not completely clear. Thus, further analyses are required to indicate the role of miR-155 in DLBCL prognosis based on clinical characteristics.

In the present study, plasma miR-155 expression levels in DLBCL

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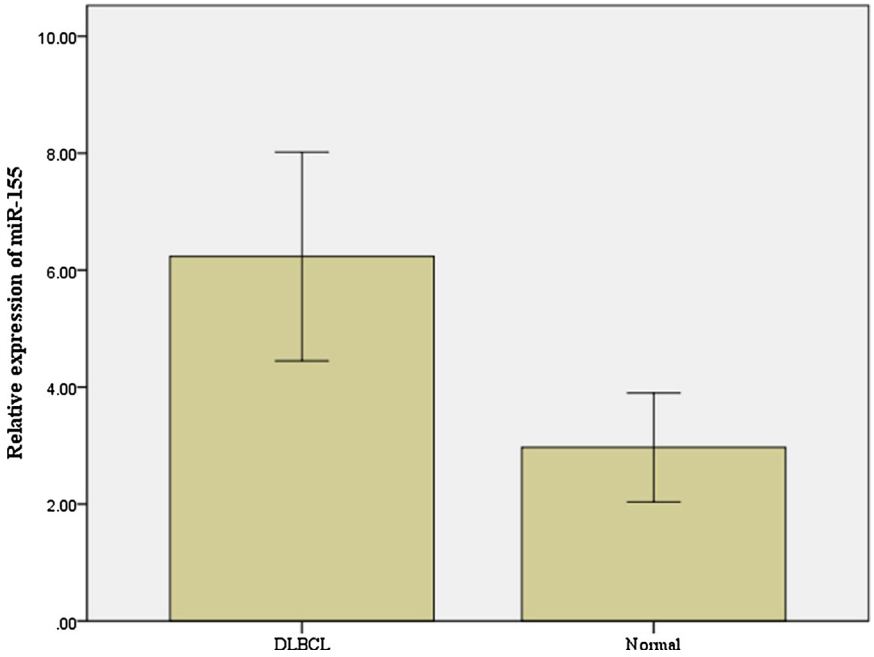

Fig. 1. Comparison of DLBCL with normal subjects. Up regulation of miR-155 in DLBCL patients. Quantitative PCR analysis of miR-155 expression level in plasma of DLBCL patients (n = 40) and normal controls (n = 38). Results are normalized to those of controls and are represented relative to expression of the U6 ($P < 0.05$).

Table 1
miR-155 expression and clinicopathological characteristics.

Clinical features	miR-155 expression levels		
	Low (n = 19)		High (n = 21)
Age			
< 60	6		11
> 60	9		14
P		0.634	
Sex			
Male			
Female			
P		0.579	
Stage			
I–II	11		12
III–IV	8		9
P		0.037	
B- symptoms			
Yes	7		9
No	11		13
P		0.048	
LDH			
Normal	9		13
Elevated	10		8
P		0.032	
IPI score			
0–2	12		15
3–5	7		6
P		0.043	

were investigated and the clinical pathologic significance and potential prognostic value for DLBCL were evaluated in the Iranian population.

2. Method

2.1. Patients and plasma sample

We assessed plasma samples obtained at the time of diagnosis of 40 DLBCL patients. All of these patients had undergone molecular and phenotypic classification with available clinical information. Moreover, 38 plasma samples were obtained from healthy individuals. The median follow-up was approximately 13.78 months (range: 2–23 months). The study was approved by the local research ethics committees and ethical permission and informed consent were obtained from all contributors.

2.2. MicroRNA isolation and quantitative real-time PCR analysis (qRT-PCR)

Total RNA was extracted using the miRNeasy Serum/Plasma Kit (Qiagen) according to the manufacturer's instructions. Next, 1000 ng of total RNA was reversed-transcribed to cDNA using a cDNA synthesis kit (Takara, Japan). The generated cDNA was stored at −20 °C for future use. qRT-PCR was carried out in a StepOnePlus Real-Time PCR System (Applied Biosystems) using the miScript® SYBR® Green PCR Kit (Qiagen) in triplicate. To normalize the expression levels of the miR-155 gene, U6 snRNA was applied as an internal control. ΔCT was calculated by subtracting the CT values of U6 snRNA from the CT values of miR-155. ΔΔCT was then determined by subtracting the average ΔCT of controls from the ΔCT of patients. The relative expression of plasma miRNA in DLBCL cases was analyzed with the $2^{-\Delta\Delta Ct}$ method using the pooled miRNA from healthy individuals as the reference [9]. RT-PCR primers: miR-155: F: 5′-GACTGTTAATGCTAATCGTGATAG-3′; R: 5′ GTGCAGG GTCCGAGGTATTC-3′, U6: F: 5′-GCGCGTCGTGAAGCGTTC-3′; R: 5′ GTGCAGGGTCCGAGGT-3.

2.3. Statistical analysis

All statistical analyses were carried out in the SPSS 20.0 software package (SPSS, Chicago, IL, USA).

3. Results

3.1. Plasma miR-155 is significantly up-regulated in patients with DLBCL

We examined the expression levels of miR-155 in plasma samples from 40 DLBCL patients and 38 healthy individuals. Based on the qRT-PCR analysis, the miR-155 expression was significantly increased in plasma samples from DLBCL cases (median expression value: 4.29, range: 1.52–27.86) compared with those of healthy controls (median expression value: 2.14, range: 0.29–10.56, $P < 0.002$) (Fig. 1).