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ORIGINAL ARTICLE

Different expressions of miR-125b and SOX30 in malignant lymphomas and their significance

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Summary

Purpose: *To explore the roles of micro RNAs (miRs)-125b and SOX30 in malignant lymphomas.*

Methods: *The correction of miR-125b targeting SOX30 was examined by the luciferase reporter assay. The expression levels of miR-125b and SOX30 were tested by in situ hybridization and immunohistochemistry.*

Results: *miR-125b was able to bind to the 3'UTR of SOX30 gene and was negatively associated with SOX30. As compared with the reactive hyperplasia lymphatic samples, the higher (miR-125b) and lower (SOX30) rate was expressed positively in the malignant lymphomas, which was related to the stage and grade of malignancy.*

Conclusions: *miR-125b can regulate SOX30 by binding to its 3'UTR. miR-125b and SOX30 act as diagnostic and therapeutic markers for malignant lymphoma.*

Key words: *malignant lymphomas, miR-125b, SOX30*

Introduction

miRNAs are non-coding RNAs that contain 19-25 nucleotides. They could incompletely or completely bind to the 3'UTR region of the target genes. miRNAs are able to inhibit the protein translation process at the post-transcriptional level. They can also regulate the expression level of the target genes so as to participate in the development of the different tumors [1,2]. Many studies have reported that miR-125b is identified as a tumor-suppressor in different cancers. miR-125b inhibits cell proliferation via regulating Sirtuin7 in hepatocellular carcinoma [3]. miR-125b is down-regulated in gastric cancer, and represses cells proliferation and invasion via controlling MCL1 [4]. The same miR contributes to fibroblast-to-myofibroblast transition and cardiac fibrosis [5]. Serum miR-125b may serve as a useful biomarker for Alzheimer's disease [6]. However, other studies have discovered that miR-125b functions as an oncogene. For example, miR-125b can enhance acute promyelocytic leukemia cell proliferation through PI3K/Akt and MAPK signaling pathways [7]. miR-125b can regulate the biological process of early B cells through modulating the target gene MAP3K11 [8]. Overexpression of miR-125b functions as a promotion factor in the development of lung cancer cells [9]. However, the underlying roles of miR-125b in malignant lymphoma were not clear.

SRY-related HMG-box 30 (SOX30) is confirmed as a target gene of miR-645 [10]. In lung cancer, SOX30 is also identified as a novel tumor suppressor promoting cell apoptosis via activation of p53 [11]. Up-regulation of SOX30 is related with favorable survival of patients with lung adenocarcinoma [12]. However, the roles of SOX30 in malignant lymphoma remain still unclear.

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This study aimed to analyze the expression levels of miR-125b and SOX30 in malignant lymphoma tissues and to explore their potential roles in malignant lymphoma, thus providing a theoretical basis for the diagnosis, treatment, and prognosis of malignant lymphoma.

Methods

Clinical samples

All of the samples were collected from the malignant lymphoma (n=174) in our hospital between January 2007 and December 2016. No patient had received chemotherapy or radiotherapy before surgery. The lymphoid tissues of the reactive hyperplasia (n=32) were included into the control group. This study was approved by the Ethics Committee of Xianning Central Hospital. Signed informed consents were obtained from all participants before the study.

Cell transfection and luciferase reporter gene activity analysis

Human lymphoma cell line (Raji) was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). At 24 hrs before transfection, 5×10^5 cells / mL were seeded into 12-well plates. By using lipofectamine 2000 transfection reagent, miR-125b mimics and SOX30-wild type (SOX30-wt) or SOX30-mutant (SOX30-mut) were co-transfected into malignant lymphoma Raji cells. At 36 hrs after transfection, the luciferase activity was analyzed by luciferase reporter kit (Promega, Madison, WI, USA).

In situ hybridization and immunohistochemical detection

miR-125b and SOX30 positive staining showed brown granules. miR-125b positive expression was located in the cytoplasm, and SOX30 positive expression was located in the nucleus, while the color intensity was higher than that of the background nonspecific staining. The results were determined by selecting randomly 5 fields of view under 40× magnification.

Statistics

SPSS 19.0 statistical software (IBM, Armonk, NY, USA) was used for statistical analyses. Percents were used to express the numerical data, and x^2 test was used for data analyses. Spearman's rank correlation test was used for correlation analysis. $P<0.05$ was considered to denote statistical significance.

Results

SOX30 was a target gene regulated by miR-125b

The online software (http://www.miRBase.org/) predicted that SOX30 was one of the target genes regulated by miR-125b (Figure 1A). To confirm the regulatory mechanism, we performed the luciferase reporter assay. Firstly, we co-transfected SOX30 3'UTR wild-type or mutant luciferase reporter plasmids with miR-125b mimics into malignant lymphoma Raji cells. The results showed that the luciferase activity was decreased in miR-125b mimics SXO30 3'UTR wild-type group, but did not change in SOX30 3'UTR mutant group (Figure 1B). These results suggested that SOX30 is a target gene regulated by miR-125b.

Higher miR-125b and lower SOX30 were expressed in malignant lymphoma tissues compared with the reactive hyperplastic lymphoid tissues

To detect the expression levels of miR-125b and SOX30 in malignant lymphoma tissues and the

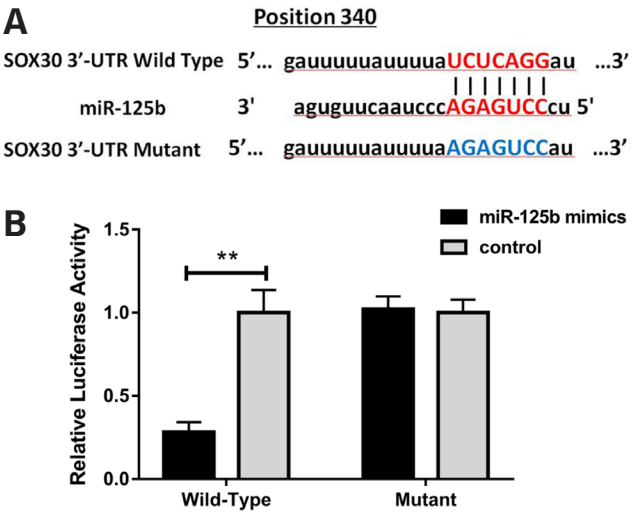

Figure 1. The binding of miR-125b to SOX30 3'UTR was predicted via online software http://www.miRBase.org/ **(A)** and was identified by using luciferase reporter assay. **(B)** Compared with the control group, the luciferase activity was decreased in miR-125b mimics SOX30 3'UTR wild-type group. These results suggest that SOX30 is a target gene regulated by miR-125b. **p<0.01 compared between miR-125b mimics and control in wild type.

Table 1. Expression of miR-125b and SOX30 in malignant lymphoma and lymphatic tissues with reactive hyperplasia

Type	*Number*	*miR-125b*				*SOX30*			
		Positive	*Negative*	x^2	*p*	*Positive*	*Negative*	x^2	*p*
Malignant lymphoma	174	116	58	14.27	0.0002	40	134	17.51	0.0000
Reactive lymphoid hyperplasia	32	10	22			19	13		

reactive hyperplastic lymphoid tissues, we used *in situ* hybridization and immunohistochemistry, respectively. The results of *in situ* hybridization indicated the positive expression rate of miR-125b in the malignant lymphoma tissues was significantly higher than that in the reactive hyperplastic lymphoid tissues ($p<0.05$, Figures 2A and B). Meanwhile, the results of immunohistochemistry revealed that the positive expression rate of SOX30 in malignant lymphoma tissues was lower than the positive expression rate of SOX30 in the lymphoid tissues of the patients with reactive hyperplasia ($p<0.05$, Figures 2C and D). The positive expression rate of miR-125b or SOX30 was statistically significant as shown in Table 1.

miR-125b and SOX30 were dysregulated in different histological types of malignant lymphoma

Furthermore, we also assessed the expression levels of miR-125b and SOX30 in the different histological types of malignant lymphoma. The positive expression rates of miR-125b in Hodgkin's lymphoma, T cell lymphoma and B cell lymphoma are shown in Table 2, and reveal no significant difference in the expression of miR-125b between histological types ($p>0.05$, Table 2). The positive expression rate of SOX30 expression in Hodgkin's lymphoma was significantly lower than that in T-cell lymphoma ($p<0.001$) and B-cell lymphoma ($p<0.001$; Table 2).

Abnormal expression levels of miR-125b and SOX30 were related to the clinical features of malignant lymphoma

Finally, we analyzed the relationship between the abnormal expression levels of miR-125b or SOX30 in malignant lymphoma tissues and the clinical features (grade of malignancy and clinical staging). There were significant differences in the expression levels of miR-125b and SOX30 among indolent lymphoma, invasive lymphoma and highly aggressive lymphoma ($p<0.01$).

In addition, significant differences were noticed in the expression levels of miR-125b and SOX30 among stage I/II, III/IV of lymphoma patients (Table 2), while no significant differences in the expressions of miR-125b and SOX30, according to sex, age and occurrence site were observed. The Spearman's rank correlation analysis demonstrated that miR-125b was negatively

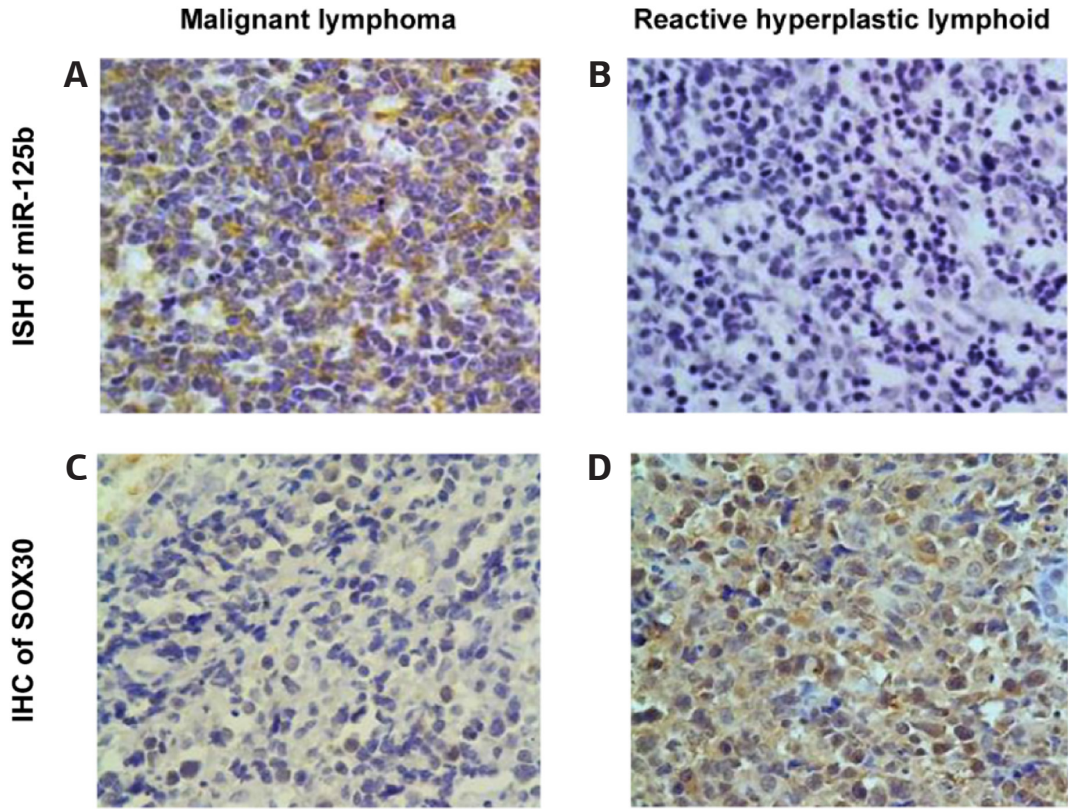

Figure 2. Expression of SOX30 in malignant lymphoma and lymphatic tissues with reactive hyperplasia via ISH **(A** and **B)** and IHC assays **(C** and **D)**. The results of *in situ* hybridization indicated the positive expression rate of miR-125b in the malignant lymphoma tissues was significantly higher than that in the reactive hyperplastic lymphoid tissues. The results of immunohistochemistry revealed that the positive expression rate of SOX30 in malignant lymphoma tissues was lower than the positive expression rate of SOX30 in the lymphoid tissues of the patients with reactive hyperplasia.

correlated with SOX30 expression in malignant lymphoma and with increased expression of miR-125b, the expression of SOX30 was attenuated (Table 3).

Discussion

miRNAs play an important role in the development of tumors and they are able to function as promotion agents of the malignant processes [13-15]. miR-192 and miR-662 can promote chemoresistance and invasiveness of squamous cell lung cancer [16]. Meanwhile some of them exhibit inhibitory effects [17-19], for example, miR-491 can suppress gastric cancer metastasis via modulating SNAIL and FGFR4 [20], and miRNA-374b can inhibit cell proliferation and induce apoptosis by the p38/ERK signaling pathway via binding to JAM-2 in cervical cancer [21]. In this study, using *in situ* hybridization, we found that the positive expression rate of miR-125b increased in the malignant lymphoma tissues compared with the reactive lymphoid tissues and that its expression increased with the grade of malignancy and clinical stage. The online software (http://www.miRBase.org/) predicted and the luciferase reporter assay identified that SOX30 was one of the target genes regulated by miR-125b. The positive expression rate of SOX30 protein in malignant lymphoma tissues was significantly higher than that in the reactive hyperplastic lymphoid group. The expression of SOX30 was down-regulated with the grade of tumor malignancy and clinical stage. This study suggests that highly expressed miR-125b may target the expression of SOX30 and participate in the development of malignant lymphoma.

Table 2. Relationship of miR-125b, and SOX30 expression with pathological parameters of malignant lymphomas

Clinicopathological features	*Number*	*miR-125b*				*SOX30*			
		Positive	*Negative*	x^2	*p*	*Positive*	*Negative*	x^2	*p*
Histological types				3.67	0.1595			7.78	0.0205
T cell	61	36	25			18	43		
B cell	92	63	29			22	70		
Hodgkin's	21	17	4			0	21		
Sex				1.54	0.2146			0.04	0.8379
Male	98	59	39			30	68		
Female	78	54	24			25	53		
Age (years)				0.01	0.9312			0.06	0.8113
<45	59	35	24			19	40		
>45	115	69	46			35	80		
Occurrence site				0.03	0.8709			0.42	0.5159
Primary nodal lymphoma	73	50	23			16	57		
Extranodal lymphoma	101	68	33			29	82		
Grade malignancy				55.29	0.0000			42.15	0.0000
Indolent	76	28	48			41	35		
Invasive	90	79	11			9	81		
Highly aggressive	18	17	1			2	16		
Clinical stage				18.85	0.0000			6.11	0.0135
I+II	118	69	49			35	83		
III+IV	56	51	5			7	49		

Extranodal lymphoma indicates lymphoma spread exteriorly of lymph node

Table 3. Relationship between the expression of miR-125b and SOX30 in malignant lymphomas

miR-125b	*SOX30*		*Total*	*p value*
	Positive	*Negative*		
Positive	11	105	116	<0.01
Negative	29	29	58	
Total	40	134	174	

One of the reasons for SOX30 down-regulation in malignant lymphoma may be that the highly expressed miR-125b inhibits the expression of the target gene SOX30 by binding to SOX30 3'UTR. The up-regulation of miR-125b expression in malignant lymphoma may be triggered by the regulation of transcription factors, genomic instability, and abnormalities of Drocer and Dicer enzymes [22-24]. Further analysis of Spearman's correlation showed that miR-125b was negatively correlated with SOX30 expression in malignant lymphoma.

Conclusions

In summary, high expression of miR-125b and low expression of SOX30 in malignant lymphoma may be regarded as important biomarkers for the development of malignant lymphoma, which provide new strategies for diagnosis and therapy of malignant lymphoma. However, there are many challenges in the exploration of miRNAs functions and regulatory mechanisms, such as improving the sensitivity and specificity of miRNA detection, enhancing the miRNA expression spectrum, providing more accurate diagnosis and prognosis by miRNA detection, and transforming miRNA basic research into clinical treatment. It is believed that miRNAs will be widely used in tumor prevention, diagnosis and treatment in the future.

Conflict of interests

The authors declare no conflict of interests.

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