Increased Malat1 Expression Predicts Poor Prognosis in Primary Gastrointestinal Diffuse Large B-cell Lymphoma

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Research Article

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Abstract

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has been involved in the pathogenesis and progression of several cancers. However, the exact effect of MALAT1 in primary gastrointestinal diffuse large B-cell lymphoma (PGI-DLBCL) has not been elucidated. This study aimed to explore the prognostic value of MALAT1 in PGI-DLBCL patients. Quantitative real-time Polymerase Chain Reaction (qRT-PCR) was performed to detect the expression of MALAT1 in 90 patients with PGI-DLBCL. MALAT1 was remarkably up-regulated in PGI-DLBCL tissues as compared to that of paired adjacent non-tumor tissues (P<0.001), and the area under the ROC curve (AUC) was 0.838. MALAT1 expression was further increased in the non-germinal center B-cell-like (non-GCB) group, advanced stage (stages IIE-IV) group and International Prognostic Index (IPI) score (3-5) group (P=0.01, P<0.001and P<0.001, respectively). Furthermore, Kaplan-Meier analysis showed that elevated MALAT1 expression was correlated with inferior Overall survival (OS) and progression free survival (PFS) in PGI-DLBCL patients (P<0.001and P<0.001, respectively), and multivariate analysis suggested that up-regulation of MALAT1 and high IPI score (3-5) were two unfavorable prognostic factors of PGI-DLBCL. In conclusion, our results demonstrates that MALAT1 might serve as a novel prognostic biomarker and an ideal therapeutic target for PGI-DLBCL patients in the future.

Introduction

The most common extranodal site of non-Hodgkin's lymphoma (NHL) is the gastrointestinal (GI) tract, constituting about 5%–20% of all NHLs and 30%–40% of all extranodal lymphoma [1]. Primary GI (PGI) lymphoma is a relatively rare disease, accounting for only 1-4% of all GI malignant tumors [2]. The most frequently involved region is the stomach, followed by small intestine, ileum, cecum, colon and rectum [3,4]. Diffuse large B-cell lymphoma (DLBCL) is the most common histopathological subtype of PGI lymphoma, following mucosa-associated lymphoid tissue (MALT) lymphoma and follicular lymphoma, respectively [5,6]. The etiology of primary gastrointestinal diffuse large B-cell lymphoma (PGI-DLBCL) remains largely elusive. Recent studies have shown that long non-coding RNAs (lncRNAs) serve as new biomarkes for diagnosis and therapy of diverse cancers, and the aberrant expression are widely associated with pathogenesis, metastasis, and tumor stage [7-9]. To date, the molecular mechanism and clinical significance of lncRNAs on PGI-DLBCL patients has not been reported.

LncRNAs are the most abundant subclass of non-coding RNA, ranging in length from 200 nucleotides to 100,000 nucleotides, which have no protein coding ability and regulate various gene expression [10,11]. Recent evidences have demonstrated that lncRNAs play important roles in normal development and diseases including cancer, which involved in various biologic processes, such as cell proliferation [10], survival [12], differentiation [13], transcription regulation [14], genomic imprinting [15], X chromosome inactivation [16]. The metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also known as nuclear enriched abundant transcript 2 (NEAT2), is one of the most widely studied lncRNAs with >8,000 nts, located on human chromosome 11q13 [17]. In 2003, MALAT1 was firstly discovered as a prognostic parameter indicating high metastatic potential and poor clinical outcomes in a study of gene expression

differences in patients with stage I and stage II non-small cell lung cancer (NSCLC) [17]. Since then, research on MALAT1 has increased year-by-year [18,19]. The abnormal expression of MALAT1 has been found in many human cancers and was involved in the occurrence and development of tumors. Many studies have revealed that MALAT1 has an elevated expression and was associated with cancer progression in various solid tumors, including hepatocellular carcinoma [20], ovarian cancer [21], cervical cancer [22] and glioblastoma [23]. Reviewing the related studies of hematological malignant tumors, the role of MALAT1 in DLBCL [24], multiple myeloma (MM) [25], mantle cell lymphoma (MCL) [26] and acute myeloid leukemia (AML) [27] also has been studied.

There is a great deal of evidence supports abnormal patterns of expression of MALAT1 has been found in a wide variety of solid tumors and hematological malignancies, but the dysregulation of MALAT1 in PGI-DLBCL patients has not been reported. Focusing on MALAT1 may provide new insights for PGI-DLBCL diagnosis, gene treatment and prognosis prediction. Therefore, this study was conducted to verify the potential effect of lncRNA MALAT1 in patients with PGI-DLBCL.

Materials And Methods

Study subjects

In this study, 90 cancer tissues (48 male and 42 female) and paired adjacent noncancerous tissues were obtained from patients who pathologically diagnosed with PGI-DLBCL in the Tianjin Medical University Cancer Institute and Hospital from 2011 to 2015. All patients did not receive radiotherapy or chemotherapy prior to surgery. All patients were randomly selected, with a balanced distribution of age and sex, no selection bias and no external validation. The PGI-DLBCL was confirmed diagnosis by two pathologists according to World Health Organization (WHO) classification system for hematologic malignancy [28]. Clinical stage of all PGI-DLBCL patients was classified according to the Lugano staging system for the Ann Arbor criteria for primary gastrointestinal non-Hodgkin’s lymphoma [29]. The clinical and pathological features of the patients were obtained by medical record review. All of the experiments were approved by the Tianjin Medical University Cancer Institute and Hospital Ethics Committee and informed consent were obtained from all patients. All the tissues were frozen at -80 ℃ for the next experiment.

RNA and complementary deoxyribonucleic acid (cDNA) preparation

Briefly, total RNA was isolated by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the standard protocols. The concentrations and purity of RNA was measured the NanoDrop 2000 (NanoDrop Technologies, Waltham, USA). Following this, total RNA was reversed-transcribed to cDNA using a cDNA synthesis kit (Takara, Japan) according to the manufacturer's instruction. The generated cDNA was stored at −20 °C for later use.

qRT-PCR

According to the target gene, qRT-PCR primers were designed and synthesized, and the corresponding cDNA was used as template to amplify MALAT1. Each sample was detected with ABI PRISM-7500 Sequence Detection System (ABI Company, Oyster Bay, NY, USA). All the reactions were run in triplicate, and averages were calculated with Applied Biosystem 7500 software. The reaction was carried out at 95 °C for 10 min, then denatured at 95 °C for 15 s, and then annealed at 60 °C for 1 min for 40 cycles. The relative expression of glyceraldehyde phosphate dehydrogenase (GAPDH) was used as internal control to regulate the expression level of MALAT1. The $2^{-\triangle\triangle Ct}$ method was used to determine the relative quantification of MALAT1 and GAPDH expression levels. The primers were as follows: MALAT1: F: 5'-AACCAGTTTCCCCAGCTTTT-3'; R: 5'- CTACATTCCCACCCAGCACT -3'; GAPDH: F: 5'-TGTGGGCATCAATGGATTTGG- 3'; R: 5'-ACACCATGTATTCCGGGTCAAT-3'.

Immunohistochemical staining and scoring

Immunohistochemical staining for CD10, B-cell lymphoma 6 (BCL-6) and Multiple myeloma antigen 1 (MUM1) in the tissue was performed. The sections were incubated with 3% H_2O_2 at room temperature for 10 minutes and then washed with phosphate buffer saline (PBS). Next, the sections were stained for the following antibodies: CD10 (ZM0283, ZSGB-BIO, CA, USA), BCL-6 (ZM-0011, ZSGB-BIO, CA, USA) and MUM1(ZM0039, ZSGB-BIO, CA, USA) at 4 ℃ overnight. After washing in PBS again, the sections were incubated with secondary antibodies (PV6000, ZSGB-BIO, CA, USA). Finally, the reactivity was visualized by diaminobenzidine (DAB). The staining intensity and percentage of positive cells were recorded. Immunohistochemical analysis was independently reviewed by at least two experienced tissue pathologists.

Treatment and response assessment

The purpose of the operation is to remove the tumor tissue and obtain the pathological tissue. According to the National Comprehensive Cancer Network (NCCN) treatment guidelines, all patients received at least 4 cycles of standard dose CHOP or R-CHOP chemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) [30]. Tumor response was confirmed through CT, type-B ultrasonic and evaluated according to the International Working Group (IWG) response criteria as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) [31]. Follow-up data were obtained from the outpatient reexamination and telephone. Overall survival (OS) is defined as the time from inclusion to death for any cause, and progressive-free survival (PFS) is defined as the time from inclusion to PD or any cause of death.

Statistical analysis

All statistical analyses were conducted using the SPSS20.0 (SPSS Inc, Chicago, USA) and GraphPadPrismV7.0 (GraphPad Software Inc) software. The relative expression level of MALAT1 in PGI-DLBCL patients and paired adjacent non-tumor tissues was analyzed by Paired Student's t-test. Group comparisons were assessed also using the unpaired Students' t-test. Pearson Chi-square test was used to analyze the relationship between the expression of MALAT1 and the clinical characteristics of PGI-DLBCL

patients. The cutoff value of tissues MALAT1 for predicting survival was determined by Receiver-operating characteristic (ROC) curve analysis. The analysis of survival curves was constructed by Kaplan-Meier method. Independent prognostic indicators were assessed in the multivariate and univariate analysis using Cox's proportional hazard model. ROC analysis was performed and the area under the ROC curve (AUC) was calculated to explore whether the levels of MALAT1 expression could be used as a diagnostic biomarker of PGI-DLBCL. Data were summarized as mean ± standard deviation (SD). In all tests, $P < 0.05$ was considered as statistically significant.

Results

Increased expression and diagnostic value of MALAT1 in PGI-DLBCL

To investigate the role of MALAT1 in PGI-DLBCL, the expression of MALAT1 in 90 cases of PGI-DLBCL tissues and normal tissues was detected by qRT-PCR. The median expression level of MALAT1 in tumor tissues was 21.8 (range 1.1 to 58.4). The median level of MALAT1 expression in adjacent non-cancer tissues was 5.8 (range 0.1 to 20.5), which was significantly lower than that in PGI-DLBCL patients' tumor tissues ($P < 0.001$, Fig. 1).

ROC curves were constructed to determine whether MALAT1 could be used as a diagnostic biomarker of PGI-DLBCL. The AUC value is 0.838 ($P<0.001$, Fig. 2). According to the analysis of ROC curve, the most significant critical point of MALAT1 was 11.4, and the sensitivity and specificity were 75.6% and 70%, respectively. Based on the cutoff value, 23 cases (25.6%) were divided into low-MALAT1group (≤11.4) and 67 cases (74.4%) were divided into high-MALAT1 group (>11.4).

Clinicopathological significance of MALAT1 in PGI-DLBCL

Ninety patients with PGI-DLBCL were recruited for the study. Sixty-nine patients (76.7%) affected region are the stomach. Using the Lugano staging system, 50 patients (55.6%) were diagnosed as advanced disease (IIE-IV stage). Sixty-nine patients (76.7%) exhibited a good performance status (0-1). All cases were stained for BCL6, CD10 and MUM1, of which 37 cases (41.1%) were GCB subtype and 53 cases (58.9%) were non-GCB subtype. Elevated LDH levels in 58 patients (64.4%), B symptoms were identified in 60 patients (66.7%) and low IPI scores (0-2) were found in 54 cases (60.0%). Of these, lymph node metastasis in 47 patients (52.2%) and bone marrow metastasis was found in 24 patients (26.7%). In total, there were 55 patients (61.1%) treated with 4-8 cycles of the R-CHOP regimens.

In order to further understand the clinical role of MALAT1 in PGI-DLBCL, we analyzed the relationship between MALAT1 expression and clinicopathological features of patients. General data of all patients in this study are summarized in Table 1. From the data, the MALAT1 level was significantly higher in patients with non-GCB pathological type ($P=0.006$), advanced Lugano stage (□E-□) ($P<0.001$), high IPI score (3-5) ($P=0.018$) and bone marrow metastasis ($P=0.024$). In contrast, no significant statistical differences were observed in the subgroup of other clinical features, such as sex, age, tumor origin, performance status, treatment and lymph node metastasis. Subsequently, we further analyzed the

expression level of MALAT1 in pathological type, Lugano stage and IPI score. The result demonstrated that MALAT1 expression was significantly associated with non-GCB subtype, advanced □E-□ stage and high IPI subgroup (P=0.01, P<0.001 and P<0.001, Fig. 3a, Fig. 3b, Fig. 3c).

Prognostic importance of MALAT1 in PGI-DLBCL

Kaplan-Meier method was used to evaluate the effect of MALAT1 levels on the prognosis of PGI-DLBCL patients. As expected, the survival analysis showed that the OS of patients with high MALAT1 expression was significantly worse than that of patients with low MALAT1 expression (P<0.001, Fig. 4a). Similarly, the PFS of the high-MALAT1 group was significantly shorter than that of the low-MALAT1 group (P<0.001, Fig. 4b). In univariate and multivariate analysis, we discovered that elevated MALAT1 expression and high IPI score had adverse effects on OS and PFS, while the non-GCB subtype was an unfavourable risk factor for OS (Table 2).

Discussion

PGI-DLBCL is an aggressive malignancy and commonly shows with some nonspecific clinical manifestations, such as gastric pain, dyspepsia, weight loss, gastric perforation, gastrointestinal bleeding and so on, possibly as a consequence of late diagnosis [32]. Additionally, PGI-DLBCL presents as a clinically heterogeneous tumor, which is generally treated with the standard CHOP or R-CHOP regimen [33]. Despite an improvement in the clinically comprehensive treatment of PGI-DLBCL, the prognosis of refractory or relapsed disease remains poor [34,35]. Hence, further researches are urgently needed to explore the molecular mechanism of PGI-DLBCL development and search for novel prognostic biomarkers and/or potential targets, ultimately making effective individual treatments to improve outcomes.

At present, researchers have found a large number of lncRNAs and proved that they were related to the occurrence and development of human diseases, especially in cancers, which could promote cancer cell proliferation, invasion and metastasis [36]. Emerging reports have shown that lncRNAs can be used as biomarkers to predict the diagnosis and prognosis of human tumors as well as therapeutic targets for tumor treatment. In this study, we discovered that the expression of MALAT1 in PGI-DLBCL was significantly higher than that in adjacent nontumor tissues, and ROC curve analysis showed that the AUC value was 0.882, indicating that MALAT1 has potential diagnostic value for PGI-DLBCL. Moreover, we observed that MALAT1 is tightly related to some clinical parameters, including pathological subtypes, Lugano staging status, IPI score and bone marrow metastasis. Through the Kaplan-Meier analysis, the results indicated that patients with high MALAT1 expression had worse OS and PFS than those with low MALAT1 expression. Combined univariate and multivariate analysis revealed that MALAT1 expression and IPI score were risk factors for OS and PFS in patients with PGI-DLBCL.

As aforementioned, MALAT1 displays a vital role in the pathogenesis and progression of diverse cancers and increasing efforts have been devoted to developing MALAT1-based cancer diagnosis and treatment. However, the mechanism of MALAT1 gene expression affecting prognosis of PGI-DLBCL remains to be

fully elucidated. Recent studies have validated that MALAT1 was involved in diverse biological processes, including cell proliferation, cell death, cell cycle, migration and invasion, via modulation of certain signaling pathways, including MAPK/ERK, PI3K/AKT, WNT/β-catenin and NF-kB, promoting tumor growth and metastasis [37-42]. MALAT1 has been reported to promote gastric cancer (GC) cell proliferation partly by recruiting SF2/ASF, a crucial member of serine/arginine-rich protein (SR) family proteins, making a potential biomarker and a therapeutic target for GC diagnosis and treatment [43]. In addition, several previous investigations have demonstrated that MALAT1 induces tumorigenesis and evolution through other molecular mechanisms, including binding to the active chromatin sites and regulating alternative splicing [44,45]. It should be noted that MALAT-1 was upregulated in DLBCL cells, and MALAT-1 silencing can decrease chemotherapy resistance by enhancing autophagy [46]. On the other hand, MALAT1 has been verified to induce DLBCL progression by regulation of miR-195 and PD-L1, then promoted epithelial-mesenchymal transition (EMT) process via Ras/ERK signaling pathway [24].

The IPI is the relatively valuable and widely used prognostic tool for almost all subtypes of non-Hodgkin's lymphoma [47]. The limitation is that the IPI evaluation system includes only a small number of clinical features and does not take into account the molecular biology of tumors. Currently, accumulating studies are performed to explore other risk factors affecting survival in PGI-DLBCL. A previous study has shown that the prognosis of patients with non-GCB is worse than that of patients with GCB [48]. Unfortunately, our study displayed no significant difference between MALAT1 expression and pathological subtypes. Ye et al. [49] discovered that MLL2 protein overexpression in PGI-DLBCL was positively related to higher clinical stage and negatively related to elderly patients (age >60 years) survival. Chen et al. [50] study demonstrated that upregulation of Mad2 might facilitate cell proliferation in PGI-DLBCL, and patients with higher Mad2 expression had inferior disease free survival (DFS). Similarly, our findings suggested that MALAT1 was increased in PGI-DLBCL and patients with elevated MALAT1 expression exhibited unfavorable outcomes, indicating MALAT1.

The present study also has some shortcomings: First, due to the limited sample, we can not get enough samples from other extranodal lymphoma patients, leading to fail to evaluate the role of MALAT1 expression in other extranodal lymphomas. Second, this study confirmed that MALAT1 had the predictive potential to PGI-DLBCL prognosis. However, owing to the limitation of samples, the present study does not independently verify the sample settings, and it is impossible to verify whether there are the same results in different samples. This needs to be further verified by expanding the sample size and including other patients with extranodal lymphoma in future studies.

In conclusion, our work presented that MALAT1 can be considered as a novel diagnostic and prognostic biomarker in PGI-DLBCL. A larger sample size is needed in the future in order to better understand the mechanisms of MALAT1 in the molecular etiology of PGI-DLBCL.

Declarations

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Conflicts of interest None.

Availability of data and material The datasets generated and analyzed during the current study available from the corresponding author on reasonable request.

Code availability Not applicable.

Authors' contributions ZHF designed the study and review the final manuscript. QZZ, CLY and WXY performed the experiment, analyzed the experimental data and wrote the manuscript. KYT helped to perform the experiment. WYF, YY, WXF, ZZG and YHL collected the clinical information of the patients and collected the specimens. GP, DTT and ZQL helped to collect the specimens. All authors read and approved the final manuscript.

Ethics approval All procedures carried out in studies involving human participants are consistent with the ethical standards of our institutional review committee and the 1964 Helsinki Declaration and its subsequent amendments or similar ethical standards.

Consent to participate All contributors provided written informed consent for participating in the entire study.

Consent for publication This manuscript is approved by all authors for publication.

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Tables

Table 1 Association between clinical characteristics and MALAT1 expression in PGI-DLBCL

Characteristics	Case NO. (n=90)	MALAT1 expression levels		*P*
		High (n=67)	Low (n=23)	
Gender				
Male	48	35	13	0.722
Female	42	32	10	
Age				
≤60	39	31	8	0.337
>60	51	36	15	
Origin				
Stomach	69	50	19	0.435
Intestinal	21	17	4	
Pathological type				
GCB	37	22	15	0.006
Non-GCB	53	45	8	
Lugano staging status				
□-□	40	22	18	<0.001
□E-□	50	45	5	
IPI score				
0-2	54	45	9	0.018
3-5	36	22	14	
ECOG				
0-1	69	49	20	0.176
2-4	21	18	3	
B symptoms				
Negative	30	20	10	0.232
Positive	60	47	13	
LDH level				
Normal	32	26	6	0.272
Elevated	58	41	17	

Treatment				
CHOP	35	27	8	0.640
R-CHOP	55	40	15	
Lymph node metastasis				
Negative	43	32	11	0.996
Positive	47	35	12	
Bone marrow metastasis				
Negative	66	45	21	0.024
Positive	24	22	2	
Abbreviations: ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B-cell–like; LDH, lactate dehydrogenase; IPI, International Prognostic Index.				

Table 2 Univariate and multivariate analyses of factors associated with PFS and OS of all patients with PGI-DLBCL

Parameters	Univariate analysis				Multivariate analysis			
	OS		PFS		OS		PFS	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Gender								
male vs. female	0.942 (0.607-1.464)	0.791	0.877 (0.654-1.362)	0.559				
Age								
≤60 vs. >60	1.127 (0.727-1.746)	0.593	1.135 (0.733-1.758)	0.570				
Origin								
stomach vs. intestinal	1.295 (0.809-2.075)	0.282	1.249 (0.779-2.002)	0.356				
Pathological type								
GCB vs. non-GCB	2.011 (1.266-3.196)	0.003	2.130 (1.346-3.373)	0.001	1.848 (1.071-3.188)	0.027	1.545 (0.903-2.642)	0.112
Lugano staging status								
I–II vs. IIE–IV	2.129 (1.293-3.507)	0.003	1.969 (1.201-3.231)	0.007	1.107 (0.691-1.773)	0.672	0.981 (0.612-1.574)	0.938
IPI score								
0–2 vs. 3–5	1.730 (1.096-2.729)	0.018	1.742 (1.106-2.746)	0.017	1.980 (1.047-3.747)	0.036	2.316 (1.253-4.282)	0.007
ECOG								
0–2 vs. 3–5	0.797 (0.504-1.261)	0.333	0.731 (0.463-1.156)	0.180				
B symptoms								
negative vs. positive	1.610 (1.007-2.574)	0.047	1.316 (0.825-2.099)	0.249				
LDH level								

normal vs. elevated	0.824 (0.534-1.271)	0.382	0.719 (0.465-1.110)	0.136				
Treatment								
CHOP vs. R-CHOP	1.058 (0.684-1.636)	0.801	1.124 (0.726-1.741)	0.599				
Lymph node metastasis								
negative vs. positive	0.710 (0.459-1.098)	0.123	0.701 (0.452-1.086)	0.112				
Bone marrow metastasis								
negative vs. positive	0.543 (0.344-0.855)	0.008	0.503 (0.320-0.791)	0.003	0.928 (0.529-1.628)	0.795	0.921 (0.529-1.603)	0.772
MALAT1 expression								
Low vs. high	1.875 (1.200-2.931)	0.006	1.780 (1.137-2.786)	0.012	1.966 (1.024-3.771)	0.042	2.252 (1.187-4.274)	0.013

ECOG, Eastern Cooperative Oncology Group; GCB, germinal center B-cell–like; LDH, lactate dehydrogenase; IPI, International Prognostic Index.

Figures

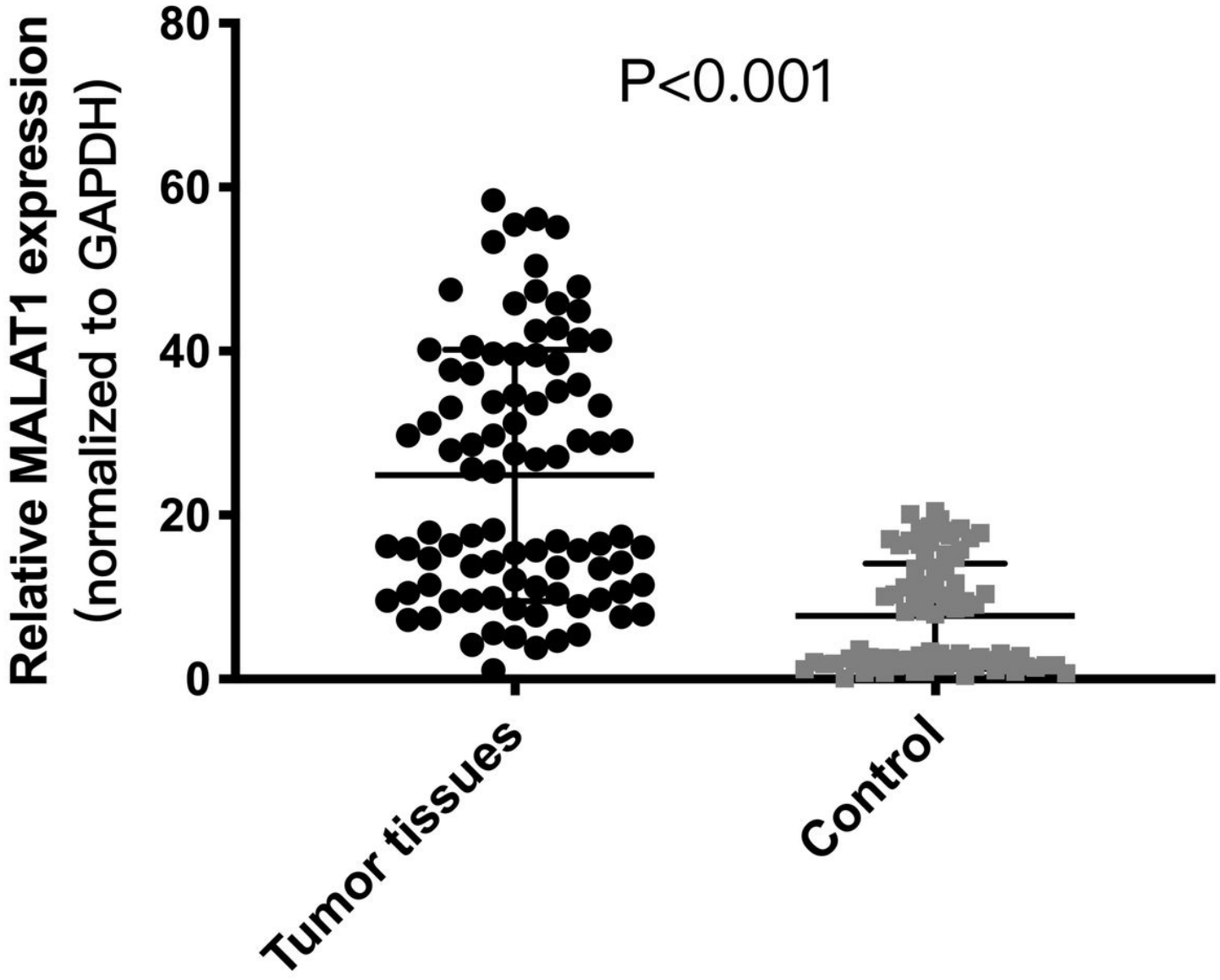

Figure 1

MALAT1 is highly expressed in PGI-DLBCL tissues. The relative expression of MALAT1 in PGI-DLBCL tissues, compared with adjacent noncancerous tissues (P<0.001). Data were presented as the mean ± SEM of three independent experiments.

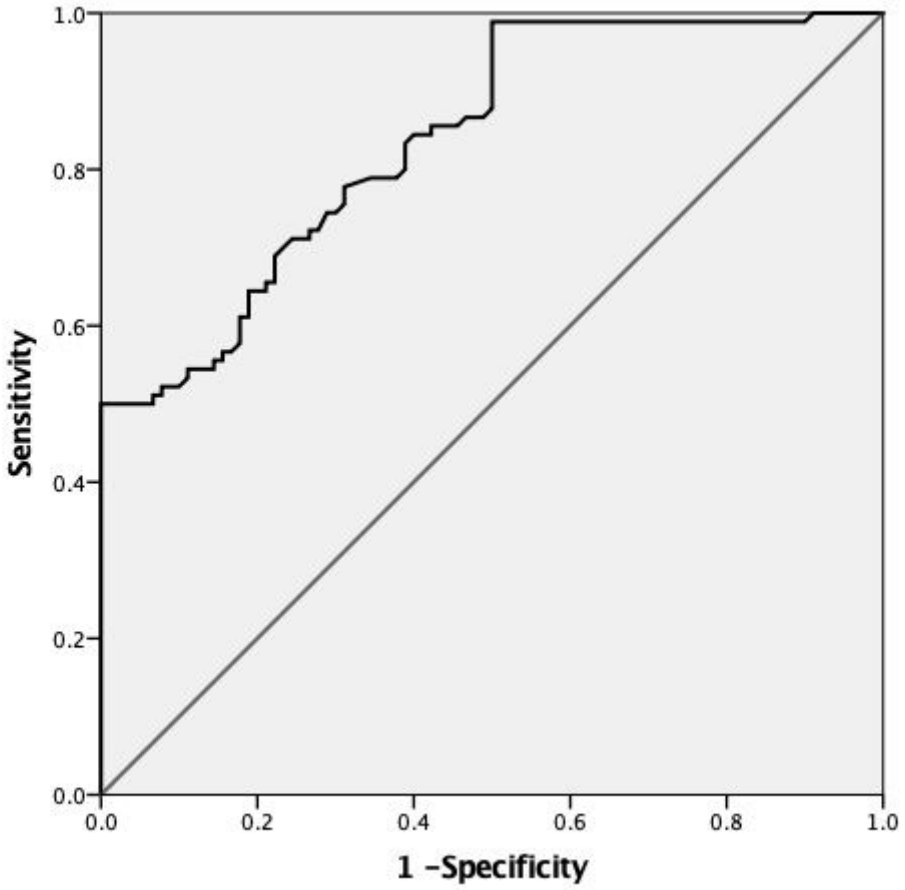

Figure 2

ROC curve analysis showing performance of MALAT1 expression to discriminate between with PGI-DLBCL tissues and normal tissues. MALAT1 showed high sensitivity and specificity for the diagnosis of PGI-DLBCL (P<0.001). The most discriminative cutoff concentration of MALAT1 was 11.4 with an AUC value of 0.838. The sensitivity and specificity were 75.6% and 70.0%, respectively.

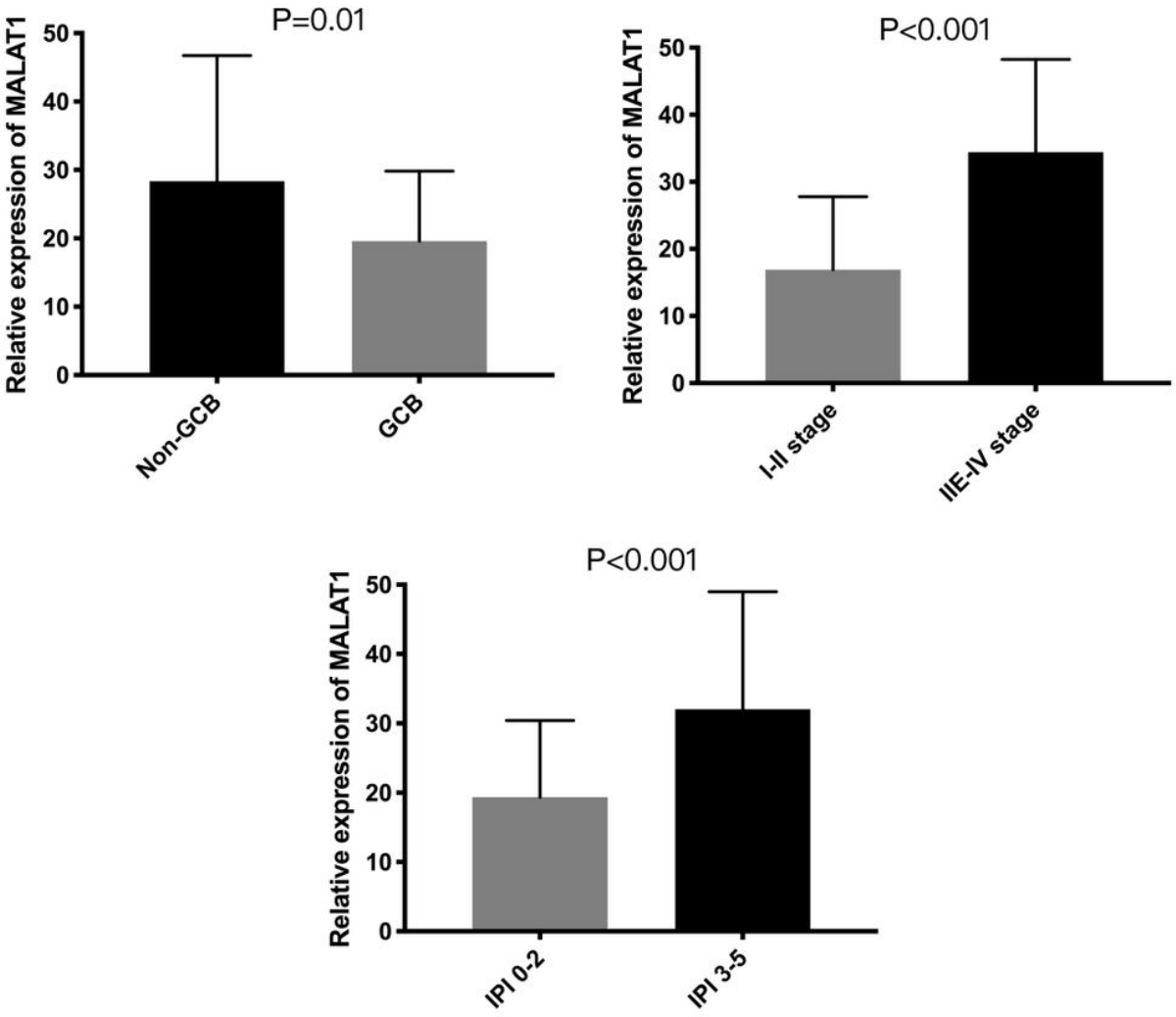

Figure 3

The expression levels of MALAT1 is associated with clinicopathological features of patients with PGI-DLBCL. MALAT1 is highly expressed in the non-GCB group (a), advanced stage (IIE–IV) group (b), and high IPI (3–5) group (c).

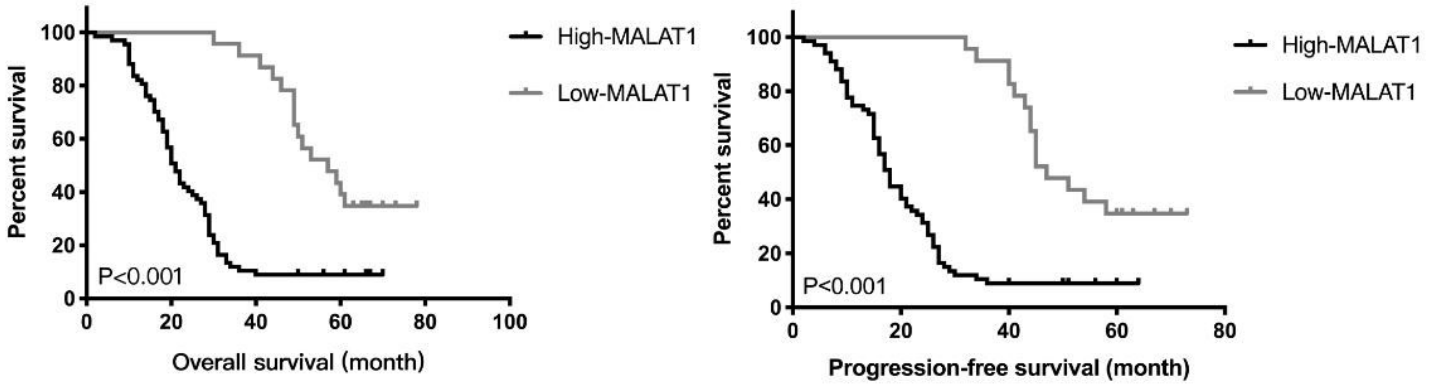

Figure 4

Kaplan-Meier analysis of overall survival and progression-free survival according to MALAT1 expression levels. Upregulation of MALAT1 showed a correlation with poor overall survival (a) and progression-free survival (b) of patients with PGI-DLBCL.