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·临床研究·

lncRNA MALAT1在弥漫大B细胞淋巴瘤患者中的表达及其临床意义

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[摘 要] **目的**:探讨长链非编码RNA(long non-coding RNA, lncRNA)肺癌转移相关转录本1 (metastasis-associated lung adenocarcinoma transcript 1, MALAT1)在弥漫大B细胞淋巴瘤(diffuse large B-cell lymphoma, DLBCL)患者血清中的表达水平及其与临床预后的相关性。 **方法**:采集2010年1月至2015年12月期间在四川省人民医院血液科确诊的、临床资料完整的82例DLBCL患者和32例淋巴结反应性增生(reactive lymphoid hyperplasia, RLH)患者的血液标本,用实时荧光定量PCR法检测两组患者血清中lncRNA MALAT1的表达水平,分析lncRNA MALAT1表达水平与DLBCL患者临床病理特征及预后的关系。 **结果**:DLBCL患者血清中lncRNA MALAT1表达水平明显高于RLH患者(7.48±0.27 *vs* 1.28±0.45,*P*<0.01)。lncRNA MALAT1的表达水平与肿瘤大小、临床分期、B症状及国际预后指数(international prognostic index,IPI)相关(均*P*<0.01)。高表达lncRNA MALAT1的患者中位无进展生存时间明显短于低表达者[(16.43±2.05) *vs* (32.01±3.20)个月,*P*<0.01],lncRNA MALAT1表达水平与IPI指数是影响DLBCL患者预后的独立因素(*P*<0.01)。 **结论**:lncRNA MALAT1在DLBCL患者中的高表达与多项临床病理参数有关,有望成为判断DLBCL患者预后的标志物。

[关键词] 弥漫大B细胞淋巴瘤;lncRNA MALAT1;临床病理特征;预后
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Expression and clinical significance of lncRNA MALAT1 in diffuse large B cell lymphoma

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[Abstract] Objective: To investigate the expression of lncRNA MALAT1 (long non-coding RNAs, metastasis-associated lung adenocarcinoma transcript 1) in the serum of DLBCL (diffuse large B-cell lymphoma) patients, and to analyze its correlation to clinical prognosis. **Methods:** The blood samples of 82 DLBCL patients and 32 RLH patients (lymph node reactive hyperplasia), who was diagnosed at Hematology Department of People's Hospital of Sichuan Province from January 2010 to December 2015 with complete clinical data, were collected for this study. lncRNA MALAT1 expression was detected by Real-time fluorescence quantitative PCR, and the relationship between lncRNA MALAT1 expression with clinical pathological features and prognosis were analyzed. **Results:** Compare with RLH patients, lncRNA MALAT1 expression was significantly increased in DLBCL patients (7.48±0.27 *vs* 1.28±0.45, *P*<0.01). In addition, lncRNA MALAT1 was significantly correlated with tumor size, clinical stage, B symptoms and International Prognostic Index (IPI) scores. The median progression free survival time (PFS) of patients with high lncRNA MALAT1 expression was significantly shorter than those with low expression ([16.43±2.05] months *vs* [32.01±3.20] months, *P*<0.01). Cox multivariate analyses verified that lncRNA MALAT1 and IPI scores were independent predictive factors for DLBCL prognosis. **Conclusion:** The high expression of lncRNA MALAT1 in DLBCL patients was correlated with multiple clinicopathological parameters., and it is expected to become a new tumor marker for DLBCL prognosis.

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弥漫性大B细胞淋巴瘤(diffuse large B cell lymphoma, DLBCL)是最常见的成人非霍奇金淋巴瘤(non-Hodgkin's lymphoma, NHL)类型，在所有NHL中DLBCL的比例约占30%, 年均发病率在7~8例/10万人[1-2]。包括环磷酰胺（cytoxan）、多柔比星（doxorubicin）、长春新碱（vincristine）和泼尼松（prednisone）的标准化疗方案(CHOP)已帮助超过40%的DLBCL患者获得长期生存。此外, 增加了利妥昔单抗（rituximab）的CHOP方案(R-CHOP)使得DLBCL患者的5年生存率提高到了接近60%。随着治疗方案的改进, DLBCL患者的预后已经取得了巨大的进步,但仍有约1/3的患者最终死于耐药或疾病复发[3-4]。长链非编码RNA（long non-coding RNA, lncRNA）参与肿瘤的发生发展过程并发挥着重要作用[5-6]，但其作用机制尚不十分清楚。肺癌转移相关转录本1（metastasis- associated lung adenocarcinoma transcript 1，MALAT1）是一种主要在核内表达且高度保守的、长度为7 kb的折叠ncRNA[7]。MALAT1在哺乳动物体内多种组织细胞内表达，参与调控肿瘤细胞的增殖、侵袭、迁移及代谢; 调控代谢相关基因的表达，在细胞G1/S期及有丝分裂过程中发挥关键作用[8]。本课题通过检测DLBCL患者血清中lncRNA MALAT1表达水平，分析其表达水平与患者临床病例特征及预后的关系，探讨其可能的作用机制及其临床意义，旨在为DLBCL的防治提供新的思路。

1　资料与方法

1.1　临床资料

采集2010年01月至2015年12月期间在四川省人民医院血液科确诊的82例DLBCL初诊患者（试验组）、32例淋巴结反应性增生（reactive lymphoid hyperplasia，RLH）患者的血液（对照组），标本采集后立即放−80℃冷冻保存备用。同时收集患者的临床资料, 包括性别、年龄、国际预后指数（international prognostic index，IPI)评分、血清乳酸脱氢酶(LDH)以及白蛋白及β2微球蛋白浓度等（表1）。DLBCL患者的诊断均根据2008年WHO公布的DLBCL诊断标准；所有患者的CD20均为阳性；均接受了R-CHOP方案化疗。采集血液标本前均告知患者并签署知情同意书，研究方案得到医院伦理委员会的批准。

1.2　实时荧光定量PCR（qPCR）检测lncRNA MALAT1的表达水平

采用TRIzol法提取血液标本中总RNA。按照逆转录试剂盒说明书进行逆转录合成cDNA。采用2× SYBR Green PCR Master Mix进行实时荧光定量PCR。取适量cDNA作为摸板，引物浓度0.4 μmol/L，15 μl体系进行扩增，每个待测样本设置3个平行样，根据MALAT1序列设计合成相应上、下游引物(上游引物为5'-CTCACTAAAGGCACCGAAGG-3'；下游引物为5'-GGCAGAGAAGTTGCTTGTGG-3')进行PCR扩增，反应条件为：95 ℃ 10 min，(95 ℃ 15 s, 60 ℃ 30 s, 72 ℃ 30 s)×40个循环。以*GAPDH*作为内参照（上游引物F: 5′-GTCAACGGATTTGGTCTGTATT-3′；下游引物R: 5′-AGTCTTCTGGGTGGC AGTGAT-3′）。采用$2^{-\Delta\Delta Ct}$方法计算目的基因的相对表达量。实验重复3次。

表1　lncRNA MALAT1表达水平与DLBCL患者临床病例特征的相关性分析

Tab.1　Correlation analysis of lncRNA MALAT1 expression and clinicopathological characteristics in DLBCL patients

Characteristic	lncRNA MALAT1 expression*		χ^2	*P*
	Low	High		
Cases(*N*=82)	39	43		
Age（*t*/a）			0.000	0.991
＜56	19	21		
≥56	20	22		
Gender			1.645	0.200
Male	19	27		
Femal	20	16		
Disease stage			10.850	<0.001
I-II	23	10		
III-IV	16	33		
Extra-nodal status			0.425	0.514
<2	8	9		
≥2	21	34		
B symptoms			35.640	<0.001
Yes	9	38		
No	30	5		
Tumor size (*d*/cm)			19.567	<0.001
<5	25	7		
≥5	14	36		
IPI score			15.705	<0.001
0-2	25	9		
3-5	14	34	0.059	0.808
GCB subtybe	18	21		
Non-GCB	21	22		
GCB				

According to the mean level of lncRNA MALAT1 (8.48) , DLBCL patients were divided into 43 cases of lncRNA high expression group（8.48）and 39 cases of low expression group（<8.48）

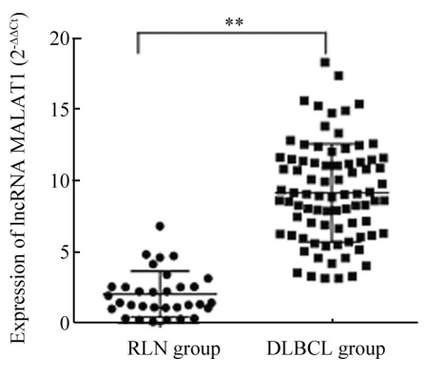
1.3 统计学处理

采用SPSS 19.0软件，计量资料以 $\bar{x} \pm s$ 表示，组间比较采用 χ^2 检验，用Spearman分析两组数据的相关性，用Kaplan-Meier绘制生存曲线，Log-Rank检验比较生存曲线的差异。以 $P<0.05$ 或 $P<0.01$ 表示差异有统计学意义。

2 结 果

2.1 DLBCL患者血清lncRNA MALAT1的表达水平明显增高

qPCR检测结果（图1）显示，DLBCL患者血清中lncRNA MALAT1的表达水平明显高于RLH患者（7.48±0.27 *vs* 1.28±0.45，t=25.540，$P<0.01$）。



**$P<0.01$

图1 DLBCL和RLH患者血清lncRNA MALAT1的表达

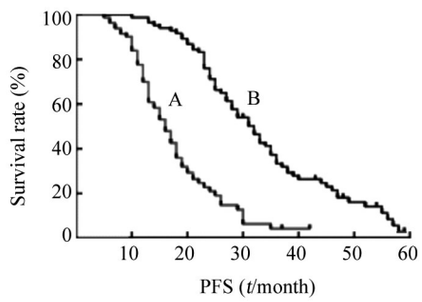
Fig.1 Expression of lncRNA MALAT1 in serum of DLBCL and RLH patients

2.2 DLBCL患者lncRNA MALAT1的表达与临床病理特征的关系

根据lncRNA MALAT1平均表达水平（8.48），将DLBCL患者分为lncRNA MALAT1高表达（≧8.48）组43例及低表达（<8.48）组39例。分析lncRNA MALAT1的表达水平与患者临床病理特征的关系的结果（表1）显示，lncRNA MALAT1的表达水平与DLBCL患者疾病分期、肿瘤大小、B症状（潮热、盗汗、体质量下降）和IPI评分正相关（均 $P<0.01$）。

2.3 lncRNA MALAT1表达水平与DLBCL患者生存期的关系

用Kaplan-Meier法统计结果（图2）发现，高表达lncRNA MALAT1的患者的中位无进展生存时间（PFS）明显短于低表达者[(16.43±2.05) *vs* (32.01±3.20)个月，χ^2=65.54，P<0.01]。



A：High lncRNA MALAT1 expression group；

B：Low lncRNA MALAT1 expression group

图3 lncRNA MALAT1的表达水平与DLBCL患者中位PFS的关系

Fig. 3 The relationship of lncRNA MALAT1 expression with the median progress free survival time in DLBCL patients

2.4 DLBCL患者预后影响因素分析

多因素Cox回归分析结果（表2）显示，lncRNA MALAT1表达与IPI评分是影响DLBCL患者预后的独立因素（$P<0.01$）。

表2 COX多因素分析影响DLBCL预后的独立因素

Tab. 1 Independent factors for DLBCL prognosis analyzed by COX multivariate analysis

Characteristic	*B*	S_x	*Wald*	*RR* (95% *CI*)	*P*
Gender	0.392	0.211	1.379	1.002(0.372～1.243)	0.079
Age(*t*/a)	0.410	0.143	0.256	1.072(0.398～1.560)	0.276
Clinical stage	1.870	0.590	1.981	1.477(0.597～1.854)	0.069
lncRNA MALAT1	3.982	1.780	3.750	4.279(2.310～9.762)	0.001
B symptoms	1.541	0.549	1.698	1.275(0.878～1.930)	0.095
Extra-nodal status	0.765	0.594	0.698	1.008(0.569～1.375)	0.174
Tumor size	1.430	0.495	0.750	1.040(0.870～1.765)	0.083
IPI score	1.892	1.947	1.773	2.280(1.716～3.950)	0.004
GCB subtybe	1.090	1.570	1.390	1.050(0.650～1.620)	0.880

3 讨 论

NHL是发病率增长最快的恶性肿瘤之一，其中DLBCL是最常见的病理类型，占所有NHL的30%~40%[9]。1994年欧美淋巴造血组织(REAL分类)指出，DLBCL是一种具有明显异质性的侵袭性肿瘤，其在

临床特征、组织形态、表观遗传学方面存在较大差异。通过联合化疗,近半数以上患者生存期(OS)得以延长, 但仍有部分患者因肿瘤进展而病死。因此, 在治疗前评估DLBCL的高危因素,采取相应的治疗方案尤为重要。

DLBCL的临床预后评估目前仍普遍采用IPI[10-12]。然而, 如何进一步评估DLBCL的预后,筛查预后不良因素是急需解决的课题,同时寻找新的治疗靶点是当前研究的热点之一。近年来的研究[13-20]表明, MALAT1与乳腺癌、胰腺癌、鼻咽癌、肝癌、等脏器的恶性肿瘤均显著相关,在上述肿瘤组织中呈高表达,且其表达水平在部分肿瘤中可能是独立的预后因素;在另外一些肿瘤的早期诊断上则显示出其潜在价值,可作为一个早期诊断的标志物, 未来有希望成为肿瘤治疗的新靶点。目前,MALAT1在DLBCL中的作用尚未见相关报道。本课题检测结果发现,DLBCL患者血清中lncRNA MALAT1表达水平明显高于RLH患者;lncRNA MALAT1的表达与肿瘤大小、临床分期、B症状及IPI指数相关(P<0.01);高表达lncRNA MALAT1的患者的中位无进展生存时间(PFS)明显短于低表达者[(16.43±2.05) *vs* (32.01±3.2)个月,P<0.01]。lncRNA MALAT1表达和国际IPI指数是影响DLBCL预后的独立因素(P<0.01)。

总之,本研究结果提示,lncRNA MALAT1在DLBCL患者血清中的高表达,其表达可能参与了DLBCL的发生发展过程。lncRNA MALAT1有望成为DLBCL患者独立预后的肿瘤标志物。

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