DOI:10.3872/j.issn.1007-385x.2018.10.011

·临床研究·

LncRNA RP11-259P1.1在小细胞肺癌组织中的表达及其临床意义

李晓华[1],代斌[2],周婷[2],周菁[2],肖贞良[2](1. 成都市第六人民医院 呼吸内科,四川 成都 610051; 2. 成都军区总医院 呼吸内科,四川 成都 610083)

[摘 要] **目的:**探讨长链非编码RNA RP11-259P1.1(lncRNA RP11-259P1.1)在小细胞肺癌(small cell lung cancer,SCLC)患者组织中的表达与患者临床病理特征的关系及其在化疗耐药中的作用。**方法:**收集2012年1月至2016年12月在成都市第六人民医院和成都军区总医院158例行支气管镜活检、穿刺活检和手术切除的SCLC患者的癌组织、42例SCLC患者手术切除的癌旁组织标本及40例正常肺组织,采用qPCR法检测癌及癌旁组织标本中lncRNA RP11-259P1.1的表达,χ^2检验分析lncRNA RP11-259P1.1表达与SCLC患者临床病理特征及化疗耐药的关系。单因素及多因素Cox回归分析lncRNA RP11-259P1.1表达与SCLC患者预后的关系。**结果:**lncRNA RP11-259P1.1在SCLC组织中的表达水平显著高于癌旁组织及正常肺组织(均P<0.01)。化疗敏感者癌组织中lncRNA RP11-259P1.1的表达水平明显低于化疗耐药者(P<0.05)。lncRNA RP11-259P1.1表达与SCLC患者的性别、年龄无关,与肿瘤分期、转移及化疗敏感性显著相关(均P<0.05);高表达lncRNA RP11-259P1.1患者的PFS及OS均显著短于低表达患者[(12.25±1.83) *vs* (22.29±1.58)个月和(23.55±1.35) *vs* (31.75±2.43)个月,均P<0.01]。lncRNA RP11-259P1.1表达、肿瘤分期及远处转移是SCLC患者独立的预后因素(均P<0.05)。**结论:**lncRNA RP11-259P1.1在SCLC组织中高表达,与SCLC患者的化疗敏感性及预后相关,可能是SCLC患者潜在的预后评估的生物标志物。

[关键词] 小细胞肺癌;长链非编码RNA; RP11-259P1.1;化疗耐药;预后
[中图分类号] R730.53; R734.2 [文献标识码] A [文章编号] 1007-385X(2018)10-1042-06

Expression and clinical significance of lncRNA RP11-259P1.1 in small cell lung cancer tissues

LI Xiaohua[1], DAI Bin[2], ZHOU Ting[2], ZHOU Jing[1], XIAO Zhenliang[2] (1. Department of Respiratory, the Sixth People's Hospital of Chengdu, Chengdu 610051, Sichuan, China; 2. Department of Respiratory, General Hospital of Chengdu Military Region, Chengdu 610083, Sichuan, China)

[Abstract] Objective: To explore the expression of long non-coding RNA RP11-259P1.1 (lncRNA RP11-259P1.1) in small cell lung cancer (SCLC) tissues and to analyze the relationship between lncRNA RP11-259P1.1 expression and SCLC clinicopathological characteristics, as well as to investigate its effect in chemoresistance. **Methods:** Tissue samples, including 158 cases of tumor tissues from SCLC patients, who underwent bronchoscopic biopsy, puncture biopsy and surgical resection, 48 cases of para-cancerous tissues and 40 cases of normal lung tissues, collected from January 2012 to December 2016 in the Sixth People's Hospital of Chengdu and General Hospital of Chengdu Military Region, were used in this study. The expression of lncRNA RP11-259P1.1 was detected by Real-time fluorescence quantitative PCR (qPCR). χ^2 test was used to analyze the relationship between the expression of lncRNA RP11-259P1.1 and the clinicopathological characteristics as well as chemotherapeutic resistance in SCLC patients. Relationship between lncRNA RP11-259P1.1 expression and prognosis of SCLC patients was analyzed by univariate and multivariate Cox regression analysis. **Results:** The expression of lncRNA RP11-259P1.1 in SCLC tissues was significantly higher than that in para-cancerous tissues and normal lung tissues (all P < 0.01). The expression of lncRNA RP11-259P1.1 in cancer tissues of chemosensitive group was significantly lower than that of chemoresistant group (P<0.05). The expression of lncRNA RP11-259P 1.1 was not correlated with gender and age, but significantly correlated with tumor stage, metastasis and chemosensitivity (all P<0.05). PFS and OS in patients with high lncRNA RP11-259P 1.1 expression were significantly shorter than those in patients with low expression ([12.25±1.83] *vs* [22.29±1.58] months, [23.55±1.35]

[基金项目] 国家自然科学基金资助项目(NO.81700081)。Project supported by the National Natural Science Foundation of China(NO.81700081)
[作者简介] 李晓华(1981-),女,硕士,主治医师,主要从事肺肿瘤的临床研究,E-mail:383152721@qq.com
[通信作者] 肖贞良(XIAO Zhenliang,corresponding author),博士,主任医师,主要从事肺肿瘤和其他呼吸疾病的临床研究,E-mail:xiaozhenliang2001@aliyun.com

vs [31.75±2.43] months, all $P<0.01$). The expression of lncRNA RP11-259P 1.1, tumor stage and distant metastasis were the independent prognostic factors in SCLC patients (all $P<0.05$). **Conclusion:** The high expression of lncRNA RP11-259P1.1 in SCLC tissues is associated with chemosensitivity and prognosis of SCLC patients, and may be a potential biomarker for prognosis evaluation in SCLC patients.

[Key words] small cell lung carcinoma (SCLC); long non-coding RNA; RP11-259P1.1; chemotherapy drug resistance; prognosis

[Chin J Cancer Biother, 2018, 25(10): 1042-1047. DOI: 10.3872/j.issn.1007-385X.2018.10.011]

小细胞肺癌（small cell lung cancer，SCLC）是一种恶性程度较高的肺部肿瘤，发病率仅占所有肺癌的15%~20%，但其诊断和治疗更加困难[1]。由于缺乏可用于临床靶向治疗的驱动基因，现有治疗手段仍然以化疗和放疗为主[2]。虽然SCLC早期对化疗敏感，但很快就会出现化疗耐药，导致复发和转移[3-5]。目前其具体机制目前尚未完全阐明，可能涉及众多耐药相关基因的调控异常。近年来人类基因组中已经发现了一类长度超过200个核苷酸的RNA分子，被称为长链非编码RNA（long non-coding RNA，lncRNA），其与多种肿瘤的发生发展密切相关[6]。课题组前期预实验研究发现，lncRNA RP11-259P1.1可能与SCLC的耐药相关，然而目前国内外尚无关于lncRNA RP11-259P1.1在SCLC中表达及意义的相关报道。本课题通过检测SCLC患者癌组织中lncRNA RP11-259P1.1的表达，分析其表达与患者临床病理特征的关系及其在化疗耐药中的作用，旨在为SCLC的诊断、治疗和预后判断提供参考依据。

1 资料与方法

1.1 研究对象

收集2012年1月至2016年12月成都市第六人民医院和成都军区总医院进行支气管镜活检或穿刺活检（116例）或手术（42例）的158例SCLC患者的组织标本（所有病例资料完整）、42例手术切除的SCLC的癌旁组织，所有患者确诊前均未接受过放化疗；同期收集的40例因各种原因导致肺外伤的正常肺组织标本作为对照组。病例纳入标准：经组织病理学诊断为小细胞肺癌，未经治疗的初治患者，有可测量和可评估的病灶作为疗效评价。病例排除标准：患者有严重的心肝肾功能损害，不能耐受放化疗；孕妇及哺乳期妇女；HIV阳性或未经处理的活动期HBV感染患者。所有标本采集前均告知患者并签署知情同意书，研究方案经所在医院伦理委员会批准。

158例患者均接受以铂类为主的化疗，其中化疗敏感者（化疗4～5个周期后肿瘤缩小30%以上或消失）63例，化疗耐药者（化疗4～5个周期后肿瘤增大30%或以上，或出现新的转移灶）95例；95例耐药者给予二线化疗方案治疗。

随访：所有患者均接受门诊或住院随访，随访起点时间为病理诊断时间，随访截止日期为2017年12月31日。随访结束时，生存64例，病死94例，没有失访病例。

1.2 主要试剂

TRIzol试剂盒、逆转录试剂盒购自美国Invitrogen公司；qPCR 2*SYBR Green PCR Master Mix试剂盒及引物购自宝生物工程（大连）有限公司。引物序列：lncRNA RP11-259P1.1上游引物为5’-TTCCCATGAGTTTTCCACTTG-3’；下游引物为5’-TCCTCAGTGAGCAGATGGAGA-3’；*GAPDH*上游引物为5’-TACATGGGCCGAGGCAAGATAA-3’；下游引物5’-TTCCATTTGTT TCC TGAGCAC-3’。

1.3 qPCR法检测SCLC组织中lncRNA RP11-259P1.1的表达

采用TRIzol提取总RNA。将提取的总RNA，按如下条件进行逆转录反应：37 ℃ 15 min的逆转录反应，98 ℃ 5 min的酶失活反应，逆转录产物于－20 ℃保存。qPCR采用2*SYBR Green PCR Master Mix，取适量cDNA作为摸板进行扩增。PCR反应条件：95 ℃ 10 min；95 ℃ 15 s，60 ℃ 30 s，72 ℃ 30 s，共40个循环。以*GAPDH*作为内参照，以$RQ=2^{-\Delta\Delta Ct}$的方法计算lncRNA RP11-259P1.1的表达量。

1.4 统计学处理

使用SPSS13.0软件进行数据分析，计量资料以$\bar{x}\pm s$表示，组间比较采用One-way ANOVA或t检验进行分析；用Chi-Square检验分析lncRNA RP11-259P1.1与各临床病理参数之间的关系；用Kaplan-Meier法绘制SCLC患者的PFS及OS曲线，分析患者生存时间的关系；单因素及多因素分析影响SCLC预后的因素。以$P<0.05$或$P<0.01$表示差异具有统计学意义。

2 结 果

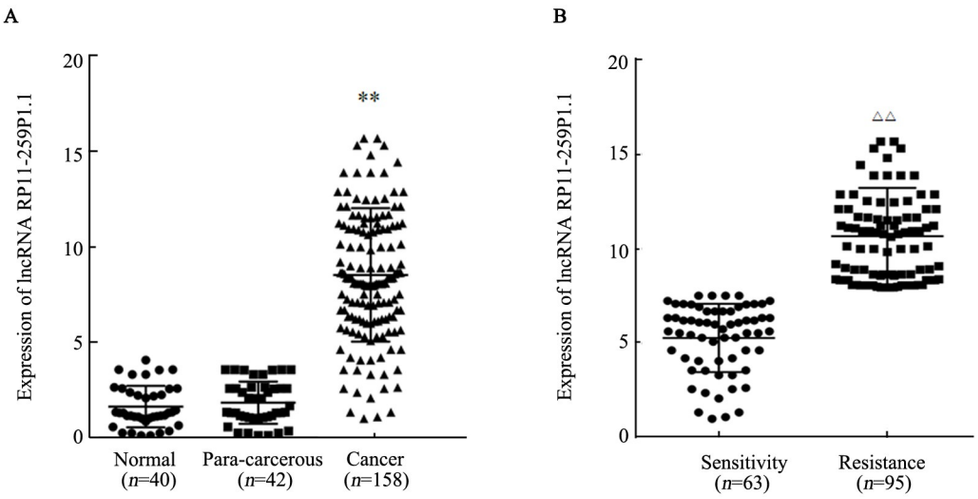
2.1 lncRNA RP11-259P1.1在SCLC组织中高表达

qPCR法检测结果（图1）显示，lncRNA RP11-259P1.1在SCLC组织中的表达水平显著高于癌旁组织和正常肺组织（$F=23.96$, $P<0.01$；图1A）；化疗耐药SCLC患者中lncRNA RP11-259P1.1的表达水平明

显高于化疗敏感者（t=14.49，P<0.01；图1B）。

2.2　lncRNA RP11-259P1.1表达与SCLC肿瘤分期、转移及化疗敏感性显著相关

经Chi-Square检验分析lncRNA RP11-259P1.1与SCLC患者各临床病理参数之间的关系，发现lncRNA RP11-259P1.1表达与SCLC患者的性别、年龄无关（均P>0.05），与肿瘤分期、转移及化疗敏感性显著相关（均P<0.05；表1）。



**P<0.01 *vs* Normal or Para-cancerous tissue group; △△P<0.01 *vs* Sensitivity group

A: Expression of lncRNA RP11-259P1.1 in SCLC tissues;

B: Expression of lncRNA RP11-259P1.1 in chemosensitive and chemoresistant patients

图1　lncRNA RP11-259P1.1在SCLC组织中的表达

Fig. 1　The expression of lncRNA RP11-259P1.1 in SCLC tissues

表1　lncRNA RP11-259P1.1的表达与SCLC患者临床病理特征的关系(n)

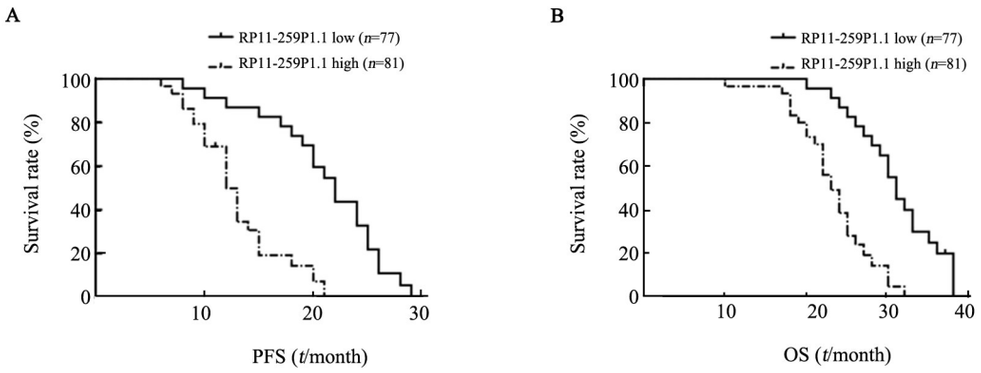
Tab. 1　Relationship between the expression of lncRNA RP11-259P1.1 and the general clinicopathological feature of SCLC patients(n)

Clinicopathological feature	Expression of lncRNA RP11-259P1.1		χ^2	P
	Low (N=77)△	High (N=81)*		
Age (t/a)				
<53	38	44	0.391	0.352
⩾53	39	37		
Gender				
Male	42	46	0.081	0.776
Female	35	35		
Disease stage				
Limited	46	20	19.937	< 0.001
Advanced	31	61		
Lymph node metastasis				
Yes	37	52	4.183	0.041
No	40	29		
Distant metastasis				
Yes	30	60	19.853	<0.001
No	47	21		
Chemotherapy				
Sensitive	40	23	9.134	0.003
Resistance	37	58		
Survival status				
Survival	44	20	17.250	< 0.001
Death	33	61		

* lncRNA RP11-259P1.1 ⩾ 8.520; △lncRNA RP11-259P1.1 < 8.520

2.3 lncRNA RP11-259P1.1高表达SCLC患者PFS和OS显著短于低表达患者

用Kaplan-Meier法绘制SCLC患者的PFS及OS曲线，分析结果（图2）发现lncRNA RP11-259P1.1高表达患者PFS显著短于低表达患者[（12.25±1.83）*vs*（22.29±1.58）个月，χ^2=22.29，*P*<0.01；图2A]；OS也显著短于低表达患者[（23.55±1.35）*vs*（31.75±2.43）个月，χ^2=21.57，*P*<0.01; 图2B]。



PFS: Progress free survival; OS: Overall survival

图2 SCLC患者的PFS（A）及OS（B）曲线

Fig. 2 The PFS (A) and OS (B) curves of SCLC patients

2.4 lncRNA RP11-259P1.1表达、肿瘤分期及远处转移是SCLC患者独立的预后因素

经单因素及多因素Cox回归分析发现，lncRNA RP11-259P1.1表达、肿瘤分期及远处转移是SCLC患者独立的预后因素（均*P*<0.05；表2）。

表2 SCLC患者预后因素分析

Tab. 2 Analysis of prognostic factors in patients with SCLC

Clinicopathological feature	Univariate analysis			Multivariate analysis		
	HR	95% *CI*	*P*	*HR*	95% *CI*	*P*
Age (*t*/a) (≥ 53 *vs* < 53)	1.002	0.820 - 1.410	0.270			
Gender (Male *vs* Female)	1.019	0.770 - 1.640	0.210			
Disease stage (Advanced *vs* Limited)	2.830	1.370 - 6.890	0.001	2.240	1.350 - 6.320	0.001
Lymph node metastasis (Yes *vs* No)	2. 70	1.350 - 7.120	0.001	1.470	0.940 - 3.060	0.060
Distant metastasis (Yes *vs* No)	2.570	1.630 - 5.850	0.001	2.140	1.450 - 5.480	0.001
Chemotherapy (Resistance vs Sensitivity)	3.630	2.080 - 8.330	0.001	2.550	1.480 - 6.030	0.001
lncRNA RP11-259P1.1 (High *vs* Low)	4.250	1.930 - 8.750	< 0.001	3.620	2.230 - 7.870	0.001

HR: Hazard ratio; *CI*: Confidence interval

3 讨 论

肺癌是临床最常见的恶性肿瘤之一，其病死率占据恶性肿瘤病死率的第一位，其发病率和病死率也出现急剧上升的趋势[7]。肺癌分为非小细胞肺癌（non-small cell lung cancer，NSCLC）和SCLC。与NSCLC相比，SCLC的生物学行为明显不同，具有低分化、高侵袭性的潜能，临床表现为迅速的癌细胞增殖并出现早期转移，患者平均5年OS率低于10%[8]。目前SCLC发生发展的潜在驱动基因和分子机制尚

不清楚，缺乏有效的针对SCLC的靶向药物，临床治疗上仍以化疗及放疗为主[9]。虽然化疗早期呈现出较好的初始反应，但化疗耐药很快出现，导致SCLC的治疗效果极不理想[10]。

近年来的研究发现，lncRNA参与调控多种类型肿瘤细胞的生物学过程，越来越多的lncRNA在基因调控等方面的功能被揭示出来[11-13]。lncRNA浆细胞瘤变异易位基因1（plasmacytoma variant translocation 1，*PVT1*）的上调是SCLC患者预后较差的标志物，参与调控SCLC细胞的侵袭和迁移[14]。lncRNA HOXA远端转录本（HOXA transcript at the distal tip，*HOTTIP*）通过富集miR-216a增加B淋巴细胞瘤-2基因（B-cell lymphoma-2，*BCL-2*）的表达，诱导SCLC的化疗抵抗[15]。lncRNA HOTTIP的表达与SCLC的疾病进展和预后相关[16]。lncRNA的HOX转录反义RNA（HOX transcript antisense RNA，lncRNA HOTAIR）通过调控HOXA1的甲基化影响SCLC的化疗耐药[17]。lncRNA牛磺酸上调基因1（taurine upregulated gene 1，*TUG1*）通过果蝇zeste基因增强子同源物2（enhancer of zeste homolog2，*EZH2*）调控LIM激酶2B（LIM kinase 2B，*LIMK2B*）的表达影响SCLC细胞增殖和化疗药物耐药[18]。lncRNA结肠癌相关转录因子2（colon cancer-associated transcript 2，*CCAT2*）作为癌基因，促进SCLC细胞增殖和转移，可作为SCLC预后不良的一个指标[19]。

本课题组前期通过lncRNA芯片发现，lncRNA RP11-259P1.1在SCLC耐药细胞株中的表达高于化疗敏感细胞株，提示lncRNA RP11-259P1.1可能与SCLC的发生和发展有关。本研究发现，SCLC组织中lncRNA RP11-259P1.1的表达水平显著高于癌旁组织及正常肺组织；化疗敏感患者组织中lncRNA RP11-259P1.1的表达水平显著低于化疗耐药患者；lncRNA RP11-259P1.1表达与患者的性别、年龄无关，而与肿瘤分期、转移及化疗敏感性相关，高表达lncRNA RP11-259P1.1患者的PFS及OS均明显缩短；lncRNA RP11-259P1.1表达是SCLC患者独立的预后因素。

本研究结果提示，lncRNA RP11-259P1.1参与调控SCLC的发生和发展，可能作为SCLC患者潜在的疗效及预后评估的生物标志物。然而，影响SCLC化疗敏感性及预后的分子机制是复杂的，仍需要深入研究。

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［收稿日期］ 2018-07-20 ［修回日期］2018-09-08
［本文编辑］ 党瑞山