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·临床研究·

lncRNA RP11-259P1.1在食管鳞状细胞癌中的表达及其与临床预后的关系

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[摘 要] **目的:** 探讨 lncRNA RP11-259P1.1 在食管鳞状细胞癌(esophageal squamous cell cancer,ESCC)中的表达水平及其与患者临床病理特征和预后的关系。**方法:**收集2012年1月1日至2016年12月31日西南医科大学附属医院行手术切除的130例ESCC原发病灶及对应癌旁组织标本、人ESCC细胞株KYSE510、Eca109及食管上皮细胞株Het-1A,采用实时荧光定量PCR法检测lncRNA RP11- 259P1.1在ESCC组织及细胞中的表达水平,分析其在组织中表达水平与患者临床病理特征及预后的关系。**结果:** lncRNA RP11-259P1.1在ESCC细胞及组织中表达水平显著高于正常管上皮细胞及癌旁组织(均P<0.01)。lncRNA RP11-259P1.1高表达与肿瘤大小、肿瘤分期、淋巴结转移及术前放化疗(CRT)后肿瘤缓解明显相关(均P<0.05), 与患者年龄、性别、肿瘤部位、吸烟状况等无关(均P>0.05)。在63例术前接受新辅助CRT治疗患者中,低表达lncRNA RP11-259P1.1患者与高表达患者比较:(1)病理完全缓解率明显升高(60.00% *vs* 21.21%,P<0.01);(2)术前CRT的疗效更佳(P<0.05);(3)中位无进展生存时间和生存时间均明显延长[(28.00±2.47) *vs* (17.00±1.90)个月,(41.57±2.45) *vs* (30.00±2.55)个月;均P<0.01]。lncRNA RP11-259P1.1为ESCC患者独立的预后因素(P<0.05)。**结论:** lncRNA RP11-259P1.1可能是ESCC潜在的预后和术前CRT疗效评价的分子标志物,低表达lncRNA RP11-259P1.1的患者术前给予CRT疗效更佳。

[关键词] 长链非编码RP11-259P1.1;食管鳞状细胞癌;临床病理特征;预后因素
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Expression of lncRNA RP11-259P1.1 in esophageal squamous cell cancer and its correlation with clinical prognosis

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[Abstract] Objective: To investigate lncRNA RP11-259P1.1 expression in esophageal squamous cell carcinoma (ESCC) and to explore its correlation with clinicopathological features and prognosis. **Methods:** 130 cases of primary ESCC cancer tissues and the corresponding paracancerous tissues resected during surgery from January 1, 2012 to December 31, 2016 at the Affiliated Hospital of Southwest Medical University, as well as human ESCC cell lines KYSE510, Eca109 and esophageal epithelial cell line Het-1A were collected for this study. The method of quantitative real-time polymerase chain reaction (qPCR) was used to measure lncRNA RP11-259P1.1 expression in specimens and cells, and the association between lncRNA RP11-259P1.1 expression in speciments and clinicopathological features or prognosis was analyzed. **Results:** The expression of lncRNA RP11-259P1.1 in esophageal cancer cells and cancer tissues was significantly higher than that in normal esophageal epithelial cells and para-cancerous tissues (all P<0.01). High expression of lncRNA RP11-259P1.1 was significantly associated with tumor size, TNM stage, lymph node metastases and chemoradiotherapy sensitivity (CRT) (P<0.05), but not associated with ages, gender, tumor location, smoking (P>0.05). In 63 cases of patients receiving neoadjuvant radiochemotherapy (CRT+S), by comparing patients with high lncRNA RP11-259P1.1 expression and low expression, (1) pathological complete remission (pCR) rate in low expression patients was significantly increased (60% *vs* 21.21%, P<0.05); (2) the treat-

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[优先发表]

ment efficacy of pre-operative CRT in patients with low expression was better (P<0.05); (3) median progress-free survival (PFS) and overall survival (OS) of patients with low expression was significantly prolonged ([28.00±2.47] months *vs* [17.00±1.90] months, P<0.01; [41.57±2.45] months *vs* [30.00±2.55] months, P<0.01). lncRNA RP11-259P1.1 can be used as a prognostic factor for patients with esophageal cancer (P<0.05). **Conclusion:** The expression of lncRNA RP11-259P1.1 could serve as a potential molecular marker to predict the prognosis and pre-operative CRT treatment efficacy of ESCC patients, and patients with low lncRNA PR11-259P1.1 expression might achieve a better treatment efficacy from pre-operative CRT.

[Key words] lncRNA RP11-259P1.1; esophageal squamous cell cancer(ESCC); clinicopathologic feature; prognostic factor

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食管鳞状细胞癌(esophageal squamous cell cancer,ESCC)的病死率在全球范围内居恶性肿瘤的第6位,我国为ESCC的高发地区[1]。目前ESCC的主要治疗方法为放疗、化疗和手术切除,由于80%以上ESCC患者确诊时已属中晚期,采用手术、放化疗等手段相结合的疗效较差[2]。lncRNA是一类长度大于200 nt的非编码RNA,其在恶性肿瘤的发生发展中起重要作用,与多种癌症的生物学过程有关[3]。虽然基因组分析发现了大量的lncRNA,但目前只有少部分lncRNA被用于研究,这些lncRNA具有作为肿瘤诊断、预后分子标志物的潜力,其表达水平与多种肿瘤的临床病理特征以及预后相关[4-6]。本课题组前期通过芯片检测lncRNA RP11-259P1.1在ESCC癌组织及癌旁组织中的差异表达,发现其在癌组织中的表达是癌旁组织中的10.30倍,提示lncRNA RP11-259P1.1参与调节ESCC的发生发展(研究结果尚未发表)。本研究通过实时荧光定量PCR(qPCR)法检测lncRNA RP11-259P1.1在ESCC细胞及组织中的表达水平,分析其表达水平与ESCC患者的临床病理特征和预后的关系,旨在探讨lncRNA RP11-259P1.1在ESCC中的可能作用及其在预后中的潜在作用。

1　资料与方法

1.1　临床资料

1.1.1　*标本来源*　收集2012年1月1日至2016年12月31日于西南医科大学附属医院行手术切除的130例ESCC原发病灶及癌旁组织(距离癌组织5 cm以上),以及患者的临床病例资料(表1)。其中男性75例,女性55例;年龄40~76岁,中位年龄59岁。63例患者术前行新辅助放化疗(CRT)后4~5周接受手术治疗(CRT+S组),67例患者给予单纯手术治疗(S组)。切取标本前均告知患者并签署知情同意书,研究方案征得医院伦理委员会批准(批准号:20111003)。

1.1.2　*治疗方法*　CRT+S组患者中有63例患者接受了相同方案的同步放化疗,具体方案:顺铂[20 mg/(m^2·d), 静脉滴注, d1-5] +5-FU [500 mg/(m^2·d),静脉滴注,d1-5];同时给予50 Gy/次放疗, 5次/周;放、化疗结束后4~5周给予手术治疗。25例患者达到完全缓解(CR),38例患者未达到临床缓解。S组中ⅡA期18例, IIB期23例,III期26例;给予单纯手术治疗,手术方式同CRT+S组。

1.1.3　*随访*　130例患者全部接受随访,中位随访时间37(3~60)个月,其中85例(65.38%)患者出现了肿瘤复发;截止2017年3月31日,存活病例57例,死亡病例73例,无失访病例。

1.2　主要试剂及细胞株、细胞培养

人食道鳞状细胞株KYSE510、Eca109及正常食道上皮细胞Het-1A均购自美国ATCC。TRIzol购自美国Invitrogen公司,AMV逆转录试剂盒、2×SYBR Green PCR Master Mix试剂盒购自大连宝生生物(TaKaRa)公司,lncRNA AC007009.1及*GAPDH*引物序列均购自大连宝生生物(TaKaRa)公司。细胞置于含10%胎牛血清的RPMI 1640培养基中,于5% CO_2、37 ℃、饱和湿度的无菌恒温箱内培养。根据不同细胞的生长情况定期换液和传代。

1.3　qPCR法检测ESCC组织及细胞中lncRNA RP11-259P1.1的表达水平

采用TRIzol方法提取ESCC细胞及组织标本中总RNA。将提取的总RNA,参照AMV逆转录试剂盒说明书进行逆转录反应,在20 μl体系中加2 μg总RNA进行cDNA合成。采用2×SYBR Green PCR Master Mix行qPCR,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,根据目标序列设计合成相应上、下游引物进行PCR扩增,上游引物为5'-TTCCCATGAGTTTTCCACTTG-3';下游引物为5'-TTCCATTTGTTTCCTGAGCAC-3'。以*GAPDH*作为内参照(*GAPDH*上游引物为5′-GGTGAAGGTCGGAGTCAACG-3′,下游引物为5′-CCATGTAGTTGAGGTCAATGAAG-3′)。PCR反应在定量PCR反应仪上进行。PCR扩增的条件是95 ℃ 10 min,(95℃ 15 s, 60 ℃ 30 s, 72℃ 30 s)×40个循环。3次独立实验后得

到的数据运用公式RQ=$2^{-\Delta\Delta Ct}$进行定量分析。

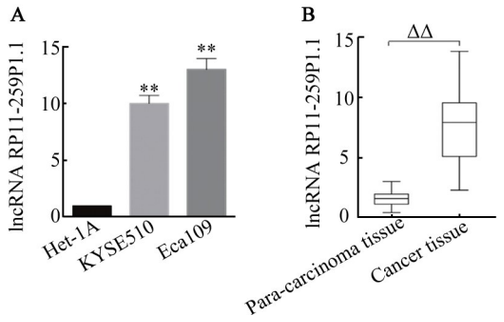
1.4 统计学处理

采用SPSS13.0统计软件，lncRNA RP11-259P1.1在ESCC细胞与正常食管上皮细胞中的差异表达采用One-way ANOVA进行分析，在癌和癌旁组织中的差异表达采用*t*检验。lncRNA RP11-259P1.1表达与各临床病理参数、与生存时间及预后的关系分别采用Chi-Square检验和Kaplan-Meier法分析；应用Cox比例风险模型分析影响ESCC的预后因素。以$P<0.05$或$P<0.01$为差异具有统计学意义。

2 结　果

2.1 lncRNA RP11-259P1.1在ESCC细胞及组织中高表达

qPCR检测结果（图1）显示，lncRNA RP11-259P1.1在ESCC细胞株KYSE510、Eca109中的表达水平显著高于正常食道上皮细胞株Het-1A（$F=19.94$，$P<0.01$）；lncRNA RP11-259P1.1在130例ESCC组织中的表达水平也显著高于癌旁组织（$t=14.59$，$P<0.01$）。



$^{**}P<0.01$ *vs* Het-1A cells; $^{\Delta\Delta}P<0.01$ *vs* Para-carcinoma tissues

图1 lncRNA RP11-259P1.1在食道癌细胞(A)及组织(B)中的表达

Fig.1 lncRNA RP11-259P1.1 expression in ESCC cells (A) and ESCC tissues(B)

2.2 ESCC组织中lncRNA RP11-259P1.1表达与患者临床病理特征的关系

根据lncRNA RP11-259P1.1平均表达水平（7.60），将ESCC患者分为lncRNA RP11-259P1.1高表达组（≧7.60）73例及低表达组（<7.60）57例。分析lncRNA RP11-259P1.1的表达与患者临床病理特征的关系(表1)发现，lncRNARP11-259P1.1的高表达与肿瘤大小、疾病分期、淋巴结转移及CRT后肿瘤缓解明显相关(均$P<0.05$)；与患者年龄、性别、肿瘤部位及吸烟状况等无关(均$P>0.05$)。

表1 ESCC患者癌组织中lncRNA RP11-259P1.1的表达与临床病理特征的关系

Tab. 1 Correlation between expression of lncRNA RP11-259P1.1 in ESCC tissues and clinicopathologic characteristics of ESCC patients

Characteristics	Lever of lncRNA RP11-259P1.1		χ^2	P^b
	Low	High[a]		
Cases (*N*=130)	57	73		
Ages（*t*/a）			0.001	0.978
＜59	22	28		
≧59	35	45		
Gender			0.573	0.449
Male	35	40		
Female	22	33		
Tumor site			0.018	0.894
Neck/Upper part	28	35		
Middle/Lower part	29	38		
Tumor size（*d*/cm）			16.191	<0.001
≥5	18	49		
＜5	39	24		
Histilogical grade			11.779	0.003
G1	26	15		
G2	19	25		
G3/4	12	33		
T stages			19.162	<0.001
T1/2	40	23		
T3/4	17	50		
N stages	30	14	18.525	<0.001
N0M0	18	49		
N1M0	9	10		
M1-lyf[c] Clinical stages			8.333	0.004
I/II stage	34	25		
III stage	23	48		
Smoking condition			0.707	0.401
Yes	27	40		
No	30	33		
CRT response	30	33	9.877	0.002
pCR[d]	18	7		
No-pCR	12	26		

[a]Median expression level of lncRNA RP11-259P1.1；

[b]$P<0.05$；[c]M1-lym: Distal lymph node metastasis；

[d]pCR：Pathologic complete response

2.3 lncRNA RP11-259P1.1表达与接受CRT治疗患者临床病理特征的关系

CRT+S组中，低表达lncRNA RP11-259P1.1的患者的病理完全缓解率（pCR）明显高于高表达患者[60.00%（18/30） *vs* 21.21%（7/33），$\chi^2=9.877$，$P<$

0.01]。单因素及多因素方差分析（表2）发现，lncRNA RP11-259P1.1与ESCC患者CRT后肿瘤缓解明显相关(均P<0.05)。

表2 单因素和多因素分析影响接受CRT患者预后的影响因子

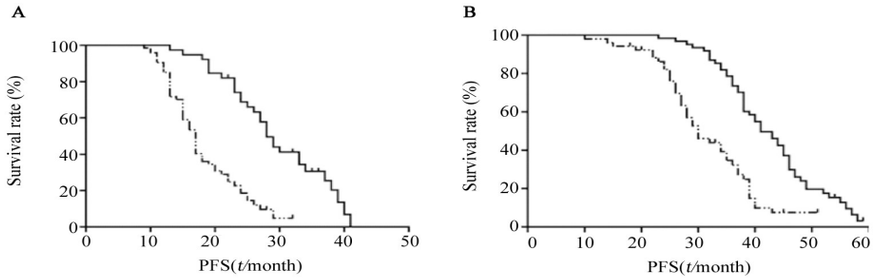
Tab. 2 Univariate and multivariate logistic regression analysis of factors associated with prognosis of patients receiving CRT

Characteristics	pCR	No-pCR	Univariate analysis		*P*	Multivariate analysis		*P*
			OR	95% CI		*OR*	95%CI	
Ages（*t*/a）			0.59	0.53-1.64	0.36	0.47	0.17-1.29	0.29
＜59	15	18						
⩾59	10	20						
Gender			0.65	0.14-1.13	0.87	0.41	0.19-1.30	0.54
Male	12	22						
Female	13	16						
Tumor site			1.12	0.89-1.94	0.75	1.09	0.76-1.56	0.38
Neck/Upper part	10	13						
Middle/Lower part	15	25						
Histilogical grade			1.38	0.56-1.64	0.63	1.24	0.38-1.46	0.44
G1	5	16						
G2	8	12						
G3/4	12	10						
T stages			1.45	0.41-3.97	0.55	1.12	0.56-1.64	0.45
I/II stage	15	18						
III stage	10	20						
N stages			1.52	0.79-3.83	0.16	1.18	0.27-2.56	0.64
N0	13	15						
N1/M1-lyf	12	23						
Clinical stages			1.82	0.75-3.75	0.089	1.39	0.70-2.36	0.10
I/II	11	14						
III	10	24						
lncRNA RP11-259P1.1			4.67	3.65-11.76	0.001	3.32	2.35-8.70	0.002
High expression	7	26						
Low expression	18	12						

pCR：Pathologic complete response; OR：Odd ratio; CI: Confidence interval

2.4 lncRNA RP11-259P1.1的表达与ESCC患者生存时间（OS）的关系

Kaplan-Meier法分析结果（图2）显示，lncRNA RP11-259P1.1高表达患者的中位无进展生存时间（PFS）明显短于低表达者[(17.00±1.90) *vs* (28.00±2.47)个月，χ^2=58.38，P<0.01；图2A）;lncRNA RP11-259P1.1低表达的患者的中位OS明显长于高表达者[(41.57±2.45) *vs* (30.00±2.55)个月，χ^2=28.17，P<0.01；图2B]。



：High expression of lncRNA RP11-259P1.1(*n*=73)；：Low expression of lncRNA RP11-259P1.1(*n*=73)

图2 lncRNA RP11-259P1.1表达与ESCC患者无进展生存(A)及总生存时间(B)的关系（Kaplan-Meier法）

Fig.2 Association between the expression of lncRNA RP11-259P1.1 and progression free survival time (A) and overall survival (B) in patients with ESCC（Kaplan-Meier survival analysis）

3 讨　论

中国是食管癌的高发区，而很多患者在确诊时已经处于中晚期，已经失去了手术切除的最佳时机[7]。虽然近年来放化疗联合治疗（包括CRT单独或作为一种手术前辅助治疗）取得了一定的进

展，但由于没有相关的放化疗敏感标志物，患者的局部复发和远处转移率仍然很高，中位OS较短[9]。lncRNA与肿瘤的形成、浸润、转移过程相关[10-14]，有研究[15]发现，lncRNA在ESCC中表达，并与患者的预后相关，lncRNA RP11-766N7.4作为抑癌基因调节ESCC细胞的上皮间质转化（epithelial-mesenchymal transition，EMT）。上调lncRNA HOTTIP的表达通过诱导EMT促进ESCC的进展[16]；lncRNA CASC9调节食管癌的迁移和侵袭[17]；循环Linc00152、CFLAR-AS1及POU3F3可作为食管癌患者术后预后不良的指标[18]；lncRNA MALAT1通过调节Cks1的表达影响食管癌患者的放疗敏感性[19]。然而，关于lncRNA RP11-259P1.1在ESCC中的功能研究目前尚未见相关报道。

本研究发现，lncRNA RP11-259P1.1在ESCC细胞中的表达水平显著高于正常食管上皮细胞和癌旁组织；lncRNA RP11-259P1.1高表达与与肿瘤大小、疾病分期、淋巴结转移及CRT后肿瘤缓解明显相关。在63例接受术前新辅助放化疗（CRT+S）的患者中，低表达lncRNA RP11-259P1.1患者的病理完全缓解率（pCR）明显高于高表达患者；低表达lncRNA RP11-259P1.1的患者术前新辅助化疗的疗效更佳；低表达lncRNA RP11-259P1.1的患者的PFS及OS均明显长于高表达患者；lncRNA RP11-259P1.1可作为食管鳞状细胞癌患者独立的预后因素。

总之，本研究结果提示lncRNA RP11-259P1.1可能是潜在的ESCC预后和CRT疗效评估的分子标志物，低表达lncRNA RP11-259P1.1的患者术前给予CRT疗效更佳。

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