Int J Clin Exp Med 2018;11(2):904-909
www.ijcem.com /ISSN:1940-5901/IJCEM0059688

Original Article

High expression of lncRNA-SChLAP1 predicts poor prognosis of colorectal cancer

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Received June 17, 2017; Accepted December 4, 2017; Epub February 15, 2018; Published February 28, 2018

Abstract: Long noncoding RNAs (lncRNAs) are an emerging class of oncogenic molecules implicated in a diverse range of human malignancies. To explore the role of lncRNA-SChLAP1 overexpression for prognostic implication in colorectal cancer (CRC), 156 cases of CRC patients meeting strict follow-up criteria and 43 cases of normal colorectal tissues were selected for qRT-PCR of SChLAP1. Correlations between SChLAP1 expression and clinicopathological features of CRC were evaluated using Chi-square tests, survival rates were calculated using the Kaplan-Meier analysis, and the relationship between prognostic factors and patient survival was analyzed using Cox proportional hazard analysis. The results showed that the levels of SChLAP1 were significantly upregulated in CRC than in non-tumor tissues. SChLAP1 high-expression correlated to differentiation and stage of CRC, and also related to low disease-free survival and overall survival rates. Further analysis using a Cox proportional hazard regression model revealed that SChLAP1 expression emerged as a significant independent hazard factor for the overall survival rate of patients with CRC. In conclusion, SChLAP1 plays an important role in the progression of CRC, and SChLAP1 may potentially be used as an independent biomarker for prognostic evaluation of SChLAP1.

Keywords: SChLAP1, colorectal cancer, prognosis, survival analysis

Intriduction

Colorectal cancer (CRC) is the third most common cancer and fourth leading cause of cancer-associated mortality worldwide [1]. Despite advances in surgical procedures and adjuvant chemotherapy, 20-25% of patients still experience relapse following curative surgery [2]. The Union for International Cancer Control (UICC) tumor node metastasis (TNM) staging system [3] is currently the most reliable indicator of patient prognosis and is widely used amongst practitioners. However, there are differences in patient prognosis even within the same TNM stage. Therefore, more reliable markers are required to improve predictions of cancer recurrence and patient survival.

Long non-coding RNAs (lncRNAs) are RNA species >200 bp in length that are frequently polyadenylated and associated with transcription by RNA polymerase II. lncRNA-mediated biology has been implicated in a wide variety of cellular processes and in cancer, lncRNAs are emerging as a prominent layer of transcriptional regulation, often by collaborating with epigenetic complexes. SChLAP1 is a novel lncRNA that is required for development and progression of prostate cancer. Prensner report that, SChLAP1, an lncRNA that is highly overexpressed in a subset of prostate cancers and associated with lethal disease, is involved in tumor cell invasion and metastasis [4]. In a limited number of samples, Rohit's preliminary data indicate that SChLAP1 expression levels can be detected in formalin-fixed, paraffin-embedded (FFPE) tissue sections by RNA in situ hybridization (ISH), suggesting potential utility of SChLAP1 as a tissue-based prostate cancer biomarker [5]. Recently, Zhang et al. found that the expression of SChLAP1 in bladder cancer was determined using real-time qPCR. Bladder cancer T24 and 5637 cells were transfected with SChLAP1 siRNA or negative control siRNA. Cell proliferation, apoptosis and migration were determined using CCK-8 assay, flow cytometry analysis and wound healing assay, respectively [6].

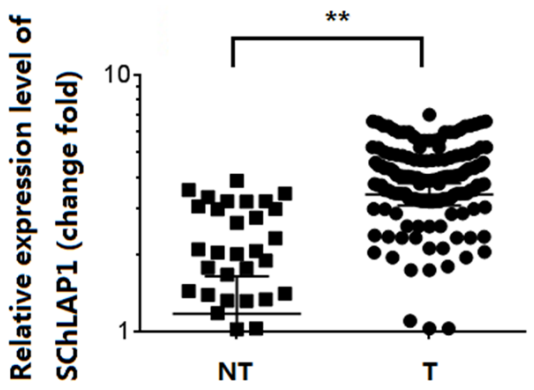

Figure 1. qRT-PCR analysis of SChLAP1 expression in CRC (T) and noncancerous tissue (NT) samples. Experiments were performed in triplicate for each case. SChLAP1 mRNA expression levels were significantly higher in CRC compared with noncancerous tissues (**P<0.01).

However, the role of SChLAP1 in prognostic evaluation and its relationship to survival in CRC is unknown, which impelled us to study the function of SChLAP1 in CRC.

Materials and methods

Ethics statement

This study complied with the Helsinki Declaration and was approved by the Human Ethics and Research Ethics committees of Eastern Liaoning University in China. Through the surgery consent form, patients were informed that the resected specimens were stored by the hospital and potentially used for scientific research, and that their privacy would be maintained. Follow-up survival data were collected retrospectively through medical-record analyses.

Clinical samples

Fresh samples from 156 cases of routinely processed CRC meeting strict follow-up criteria were selected at random from patients undergoing surgery between 2007 and 2009 at the Department of Pathology and Tumor Tissue Bank, Eastern Liaoning University. Pathological parameters, including age, gender, tumor size, TNM stage, CEA level, disease-free, and overall survival data, were carefully reviewed. Patients aged between 31 and 76 years, with a mean age of 51.6 years. The male to female ratio was 114:42. Tumors were staged according to the 6th edition of the American Joint Committee on Cancer [7]. Of the 156 CRC samples, 87 were determined as early-stage (I-IIA), and 69 as late-stage (IIB-IV). No patients had received chemotherapy and radiotherapy before surgery. By March 2017, 89 patients had died and 66 patients remained alive. 43 cases of adjacent non tumor tissues were enrolled as controls.

RNA extraction and qRT-PCR

Total RNA from fresh tissues was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was synthesized by PrimeScript reverse transcriptase (Takara Biotechnology, Dalian, China) and oligo (dT) following the manufacturer's instructions. To examine expression, real-time PCR was performed with a Bio-Rad sequence detection system according to the manufacturer's instructions using a double-stranded DNA-specific SYBR Premix Ex TaqTM II kit (Takara Biotechnology). Double-stranded DNA-specific expression was tested using the comparative Ct method using 2-ΔΔCt. The SChLAP1 primers were: 5'-CGGAGAGGATGGGCTCTGGCATT-3', 5'-AGGGACCCTTCAGGGTGGCTG-3; GAPDH was used for an internal control: 5'-CCCATCACCATCTTCCAGGAG-3', 5'-GTTGTCATGGATGACCTTGGC-3'. All assays were performed in triplicate and repeated at least three times.

Statistical analyses

Statistical analyses were performed using SPSS 19.0. Correlation between SChLAP1 expression and clinicopathological characteristics were evaluated using χ^2 tests and Fisher's exact tests. The disease-free and overall survival rates after tumor removal were calculated using the Kaplan-Meier method, and differences in survival curves were analyzed using log rank tests. Multivariate survival analysis was performed on all significant characteristics measured by univariate survival analysis with the Cox proportional hazard regression model. A P-value of <0.05 was considered statistically significant.

Results

SChLAP1 is upregulated in CRR

The qRT-PCR was used to measure SChLAP1 expression levels in a total of 156 patients with

Table 1. Relationship between SchLAP1 high-expression rate and the clinicopathological features in 156 cases of CRC

Characteristic	No. of cases	High-expression rate (%)	OR (95% CI)	*P* value
Gender			1.233 (0.602-2.529)	0.568
Male	114	52 (45.6%)		
Female	42	17 (40.8%)		
Age (years old)			1.365 (0.714-2.610)	0.348
≥52	93	44 (47.3%)		
<52	63	25 (39.7%)		
Tumor size (cm)			1.355 (0.700-2.622)	0.369
≤5	101	42 (41.6%)		
>5	55	27 (49.1%)		
Location			1.801 (0.944-3.437)	0.074
Colonic and ileocecal	87	44 (50.6%)		
Rectal	69	25 (36.2%)		
Differentiation			2.989 (1.499-5.961)	0.002**
Well	60	17 (28.3%)		
Poorly and moderately	96	52 (54.2%)		
Lymph node metastasis			1.324 (0.701-2.499)	0.388
-	88	36(40.9%)		
+	68	33(48.5%)		
Serosal invasion			1.828 (0.962-3.472)	0.065
-	74	27 (36.5%)		
+	82	42 (51.2%)		
Stage			2.470 (1.290-4.730)	0.006**
I-IIA	87	30 (34.5%)		
IIB-IV	69	39 (56.5%)		
CEA			1.070 (0.565-2.026)	0.837
Normal	67	29 (43.3%)		
Increased	89	40 (44.9%)		

Statistical analyses were performed using χ^2 tests and Fisher's exact tests. *P<0.05, **P<0.01.

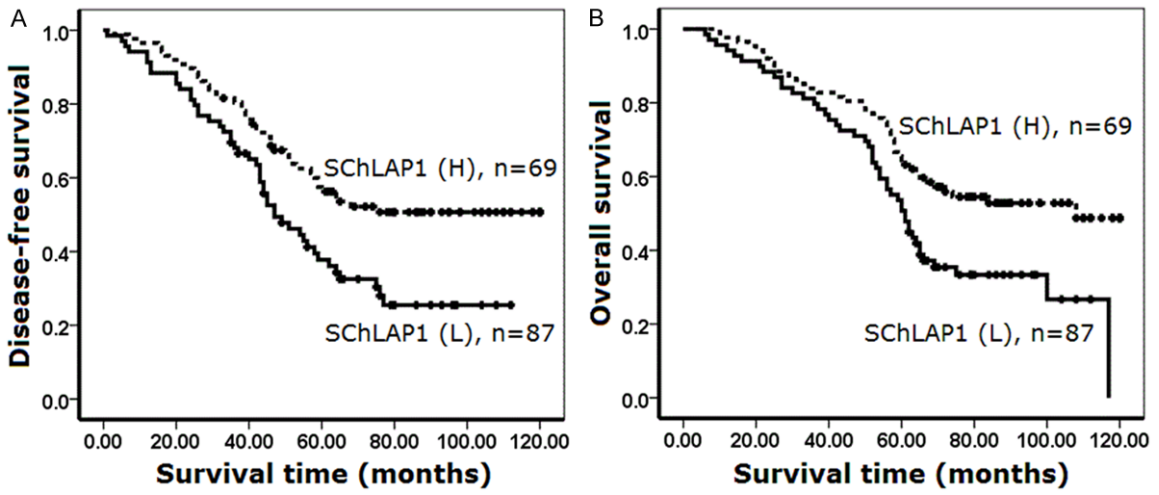

Figure 2. Kaplan-Meier analysis of disease-free and overall survival rates in 156 CRC patients in relation to SChLAP1 expression. A: CRC patients with SChLAP1 high-expression (H) had lower disease-free survival rate than those with SChLAP1 low-expression (L) (Log-rank =13.318, *P*=0.000). B: CRC patients with SChLAP1 high-expression had lower overall survival rate than those with SChLAP1 low-expression (Log-rank =10.910, *P*=0.000).

Table 2. Univariate and multivariate survival analysis of clinicopathological factors for the overall survival rate of 156 patients with CRC

Factors	Univariate		Multivariate	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Tumor size	1.632 (1.167-2.282)	0.004**	1.425 (1.005-2.022)	0.047*
Diff.	1.674 (1.199-2.336)	0.002**	1.353 (0.951-1.927)	0.093
Stage	2.445 (1.706-3.504)	0.000**	2.047 (1.406-2.979)	0.000**
SI	1.902 (1.370-2.642)	0.000**	1.595 (1.137-2.237)	0.007**
SchLAP1	1.715 (1.236-2.379)	0.001**	1.424 (1.013-2.003)	0.042*

Statistical analyses were performed using Cox proportional hazard regression model. *P < 0.05, **P < 0.01.

CRC. The results showed that SChLAP1 had higher expression in CRC tissues than that in 43 cases of normal tissues (*P*<0.01, **Figure 1**).

Correlation between SChLAP1 expression and clinicopathological factors of CRC patients

Then, we analyzed the correlation between SChLAP1 expression and clinicopathological factors of CRC patients. The median expression level of SChLAP1 was used as a cut-off point to divide all 156 patients into two groups (high and low). **Table 1** summarizes the association between SChLAP1 expression and clinicopathological parameters in CRCs. The results showed that high expression of SChLAP1 was significantly associated with differentiation (*P*=0.002), and TNM stage (*P*=0.006). However, no significant differences about other characteristics of patients were found.

Upregulation of SChLAP1 confers poor prognosis in patients with CRC

Using Kaplan-Meier method and log-rank test, the disease-free and overall survival durations was significantly shorter in patients with high SChLAP1 high-expression compared to those with SChLAP1 low-expression (*P*<0.001, **Figure 2**). Moreover, multivariate survival analysis was performed using the Cox proportional hazards model for all the significant variables found with univariate survival analysis. The results suggested that SChLAP1 overexpression also emerged as a significant independent prognostic factor in prognosis of CRC (HR: 1.424, 95% CI: 1.013-2.003, *P*=0.042), as well as tumor size (HR: 1.425, 95% CI: 1.005-2.022, *P*=0.047), clinical stage (HR: 2.047, 95% CI: 1.406-2.979, *P*<0.001), and Serosal invasion (HR:1.6595,95%CI:1.137-2.237,*P*=0.007)(**Table 2**).

Discussion

LncRNAs have been associated with several functions, including epigenetic regulation of gene expression by acting as regulatory factors in cis, as well as in trans by involvement in chromatin remodeling [8, 9]. Additionally, direct binding to active androgen receptor (AR) and recruitment of additional factors for AR-mediated gene expression has been reported [10]. However, a recent study found contradicting evidence for these findings and thus further research is required to clarify lncRNA involvement in AR activity [11]. Still, many functional relationships of lncRNAs as well as their tissue-specific regulation remain unclear. Currently, lncRNAs are gaining more interest as potential biomarkers for various malignant diseases, due to their highly tissue-specific expression profiles [12].

Regarding cellular biology, SChLAP1 is a lncRNA, which is an emerging class of RNA molecules that do not encode for a protein. Moreover, SChLAP1 is an essential mediator of aggressive disease processes, including tumor invasion and hematogenous spread [4]. SChLAP1 operates through transcriptional regulation via antagonism of the SWI/SNF epigenetic complex, which is responsible for the positioning of histone proteins at gene promoters [13, 14]. SWI/SNF is a well-defined tumor suppressor in numerous cancer types, including prostate cancer, and is inactivated by genetic mutation or deletion of core subunits [15, 16]. By disrupting SWI/SNF function, SChLAP1 contributes to the altered expression of hundreds-to-thousands of genes, which may facilitate the metastatic cascade globally rather than through a single signaling pathway, potentially enhancing early castrate resistance and risk of mortality [17]. Although selected SWI/SNF-associated proteins have been suggested to promote CRC proliferation, it is unclear whether these proteins are functioning in conjunction with, or independently of, endogenous core SWI/SNF enzymatic subunits [18, 19]. SChLAP1 may play an important role in the occurrence and development of tumors, and has been found to be upregulated in prostate and bladder cancers [4, 20]. However, the exact function of SChLAP1 in CRC remains unknown.

To test the levels of SChLAP1 might function as a prognostic factor and could be associated with tumor progression in human CRC, we examined SChLAP1 expression and found that SChLAP1 had higher expression in 156 CRC tissues than that in 43 cases of normal tissues. Moreover, SChLAP1 high-expression rate was significantly correlated to differentiation, and clinical stage. With regard to survival, moreover, we found that CRC patients with SChLAP1 high-expression had a lower disease-free survival rate and overall survival rate than patients with SChLAP1 low-expression. Finally, multivariate survival analysis demonstrated that SChLAP1 high-expression emerged as a significantly independent hazard factor for survival in CRC, along with clinical stage, serosal invasion, and tumor size.

In summary, the present study is the first to report that SChLAP1 is upregulated in CRC tumor tissues and is associated with differentiation and clinical stage of CRC. Furthermore, the results suggested that SChLAP1 is an independent predictor of poor overall survival of patients with CRC. Understanding the critical role of SChLAP1 in CRC may lead to the development of a novel diagnostic marker for this type of cancer. However, the molecular mechanisms by which SChLAP1 regulates the CRC cancer migration and invasion require further investigation. As this study is the first to report the biological functions of SChLAP1 in CRC cancer, further studies in a larger number of samples and investigations of the other possible mechanisms of action are required.

Acknowledgements

Supported by grants from the Projects of Science Research of Education Department in Liaoning Province (L2015189), Liaoning Science & Technology Project (2015020554), and Special Fund for Central Finance (2017).

Disclosure of conflict of interest

None.

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