

Aberrant expression of lncRNA Sox2ot is associated with advanced tumor progression and poor prognosis in patients with colorectal cancer

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Abstract

Background: Long non-coding RNA (lncRNA) plays an important role in carcinoma progression and prognosis. However, little is known about the pathological role of lncRNA Sox2ot in colorectal cancer (CRC) patients. This study sought to investigate the expression level of lncRNA Sox2ot in CRC, as well as explore its association with CRC progression and prognosis. **Methods:** lncRNA Sox2ot expression was measured in 117 paired CRC tissues and adjacent non-cancerous tissues using quantitative real-time polymerase chain reaction (qRT-PCR). Then, the relationship between lncRNA Sox2ot expression and clinicopathological characteristic of CRC patients was detected by Chi-square test. Lastly, relationship between the expression level of lncRNA Sox2ot and overall survival (OS) was analyzed using Kaplan–Meier analysis and univariate and multivariate analyses were evaluated by Cox regression models. **Results:** The expression level of lncRNA Sox2ot in CRC tissues were significantly higher than in the adjacent non-cancerous tissues ($P < 0.05$). In addition, high lncRNA Sox2ot expression was positively correlated with N stage, T stage, TMN stage, distant metastasis, histological differentiation grade and lymph node involvement in CRC patients (all, $P < 0.05$). Kaplan-Meier analysis showed that patients with high expression of lncRNA Sox2ot had a shorter OS than those with low lncRNA Sox2ot expression (log rank test, $P < 0.001$). Moreover, in univariate and multivariate Cox regression models, lncRNA Sox2ot expression was an independent prognostic factor for CRC patients. **Conclusion:** lncRNA Sox2ot over-expression may serve as an unfavorable prognosis predictor for CRC patients.

Background

Colorectal cancer (CRC), which ranks the third in the cancer morbidity and the second in the cancer mortality, is the most prevalent malignant cancer in the world [1]. Although great progresses have been made in therapy of CRC, 30-40 % patients still died of relapse and metastasis, among which liver metastasis is the leading cause of death [2-4]. Due to the chemotherapy and radiation therapy, the incidence and mortality of CRC are decreased in recent years. But the 5-year survival rate of CRC patients remains unsatisfactory due to metastasis and leading to poor outcomes [5, 6]. Therefore, it is important to identify novel bio-markers that can accurately identify the biological characteristics of tumors and improve the prognosis in patients with CRC.

Recently, many studies highlighted that a number of long non-coding RNAs (lncRNAs) play essential roles in carcinogenesis, and suggest that these genes might be used as bio-markers in carcinoma [7-9]. lncRNAs are a type of RNA molecules with length of more than 200 nucleotides (nt) and lack an open reading frame of significant length and have no capability of coding protein [10-12]. lncRNAs comprise the main-stream of transcripts and not translate into proteins [13], and also show emerging roles in the regulation of critical cellular functions, including transcriptional, posttranscriptional, and epigenetic mechanisms of gene regulation [7, 10, 14, 15].

Sox2 overlapping transcript (*Sox2ot*) was mapped on the 3q26.33 genomic site. *Sox2ot* is a lncRNA transcribed in the same orientation as *Sox2* [16]. Previous studies have reported the expression pattern

and functions of *Sox2ot* in cancers, including lung cancer [17] and hepatocellular carcinoma [18]. Moreover, Liu et al. have shown that *lncRNA Sox2ot* could promote CRC cell proliferation and motility and knockdown of *Sox2ot* suppressed cell migration and invasion [19]. However, little is known about the significance of *Sox2ot* expression and its prognosis value in CRC.

In the present study, we investigated the expression level of *lncRNA Sox2ot* in human CRC tissues, and then explored the association between *lncRNA Sox2ot* expression and clinicopathological characteristics. In addition we also explored the prognosis value of *lncRNA Sox2ot* in CRC.

Methods

Ethics statement

The study was approved by Ethics Committee of Southwest Hospital, Army Medical University before it was initiated. All patients provided written informed consent to the surgical procedures and gave permission to use resected tissue specimens for research purposes. The procedures of the study were in accordance with the ethical standards of the committee on human experimentation of the institution.

Patients and tissue specimens

We collected 117 paired CRC tissues and adjacent non-cancerous tissues from patients who underwent surgery at Southwest Hospital, Army Medical University. The diagnosis of CRC was confirmed pathologically by two independent experienced pathologists. Besides, we retrospectively reviewed the medical records of 117 patients. All patients were given questionnaires about their symptoms and medical history before taking a physical exam. Patients with a previous history of cancer or who had already received surgery, chemotherapy, or radiotherapy were excluded from this study.

After treatment, postoperative 5-year follow-up constructed at our outpatient department. During the follow-up, the patients were evaluated at the hospital or contacted by telephone or letter every 3 months in the first 3 years, every 6 months in the fourth year and annually thereafter. The following data were collected from the patients for further investigation: general information, preoperative information, details of the surgery, pathology reports, TNM stage, and results of the follow-up. For follow-up purposes, the primary end point was the overall survival (OS), defined as the time from surgery to mortality due to any cause. Adverse event was defined as tumor progression or death.

Tumor tissues and adjacent normal tissues from 117 patients were extracted and snap froze in liquid nitrogen. Then all samples were stored at -80°C until total RNA extraction.

RNA isolation and cDNA synthesis

Total RNA was extracted from all tissues samples with TRIzol[®] Reagent (Invitrogen; Carlsbad, CA, USA), following the manufacturer's instructions. The concentration and purification of isolated RNA was evaluated with a NanoDrop[®] ND-2000 Spectrophotometer (NanoDrop Technologies Inc.; Wilmington, DE,

USA), Purity was estimated with the absorbance ratio 260nm/280nm (mean ratio=1.91; range, 1.52-2.37). cDNA was synthesized from RNA with the PrimeScript™ RT reagent Kit (Perfect Real Time; TaKaRa Bio Inc.; Tokyo, Japan) according to the manufacturer's instructions and stored at -80°C for further use.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The qRT-PCR reaction of *lncRNA Sox2ot* and *GAPDH* were performed using LightCycler® 480 SYBR Green II real-time PCR system (Roche Applied Science) equipped with LightCycler® 480 software according to manufacturer's instruction. The primers for *lncRNA Sox2ot* and *GAPDH* (internal control) were listed in **Table 1**. The following cycle conditions included: 94°C 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min; finally 72°C 10 min. When the reactions were finished, expression levels of mRNA were automatically calculated using the number of cycle threshold (CT) and normalized to internal control *GAPDH*. Fold change in mRNA expression after normalization was calculated using $2^{-\Delta\Delta CT}$ method.

Statistical analysis

All the statistical analyses were performed with SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, La Jolla, CA, USA). All data were presented as mean \pm standard deviation (SD). Student's t-test was used to analyze the difference in *lncRNA Sox2ot* expression between the CRC tissues and adjacent non-cancerous tissues. The correlation between the expression levels of *lncRNA Sox2ot* and the clinicopathological features of CRC was analyzed using the Chi-square test. Survival curves for the patients were calculated using the Kaplan-Meier method. Prognostic factors were examined by univariate and multivariate analyses using Cox regression model. $P < 0.05$ was considered as statistically significant.

Results

lncRNA Sox2ot expression was higher in CRC tissues than adjacent non-cancerous tissues

We performed qRT-PCR to measure the expression of *lncRNA Sox2ot* in CRC tissues and adjacent non-cancerous tissues. As shown in **Figure 1**, the expression level of *lncRNA Sox2ot* was significantly higher in CRC tissues (1.142 ± 0.273) than in the adjacent non-cancerous tissues (0.668 ± 0.281). Besides, there was a significant difference between two groups ($P < 0.001$). These results indicated that *lncRNA Sox2ot* expression was abnormally elevated in CRC patients.

The correlations between *lncRNA Sox2ot* expression and clinicopathological characteristics of CRC patients

To further investigate whether the expression level of *lncRNA Sox2ot* was associated with the development of CRC, the relationship between its expression and clinicopathological features was assessed and summarized in **Table 2**. The results demonstrated that high *lncRNA Sox2ot* expression was

significantly associated with N stage ($P=0.002$), T stage ($P=0.001$), TNM stage ($P=0.008$), histological differentiation grade ($P=0.002$), lymph node involvement ($P=0.008$) and distant metastasis ($P=0.004$). Meanwhile, there was no significant association between *lncRNA Sox2ot* expression and age, gender, tumor size, tumor histology, primary tumor localization or Karnofsky performance status (all, $P>0.05$).

lncRNA Sox2ot expression is associated with overall survival in CRC patients

Kaplan-Meier analysis and log-rank test were performed to assess the prognostic value of *lncRNA Sox2ot* expression in CRC patients. From the curve, we observed that patients with high expression level of *lncRNA Sox2ot* had significantly shorter survival time than those with low expression level of *lncRNA Sox2ot* ($P<0.001$, **Figure 2**). In addition, we also observed that *lncRNA Sox2ot* over-expression was an unfavorable prognostic factor in CRC patients (**Table 3**). Both univariate analysis and multivariate analysis showed that high *lncRNA Sox2ot* expression ($P=0.000$, HR=3.783, 95%CI=2.119-6.756) was an independent poor prognostic factor for CRC patients.

Discussion

CRC is a highly heterogeneous disease and the third leading cause of cancer-related death worldwide [20]. Despite recent diagnostic and therapeutic advances have improved the clinical outcomes of CRC patients with early stage, a large fraction of early stage CRC patients still develop recurrence or metastasis. Moreover, there are few reliable markers available to accurately predict metastasis in early stage CRC patients, and individual adjuvant treatment remains a challenge. Therefore, finding new molecular markers for early diagnosis, prognosis and treatment of CRC is of great importance to improve the outcome of this disease.

In recent years, lncRNAs have been increasingly reported to be involved in a number of important events, such as epigenetic regulation, transcriptional regulation, post-transcriptional regulation [21] and some human diseases [22, 23]. Emerging evidence showed that lncRNAs may serve as diagnostic or prognostic bio-markers for some cancers [24]. For example, Zhang et al. investigated that up-regulation of *lncRNA MALAT1* correlated with tumor progression and poor prognosis in clear cell renal cell carcinoma [25]. Cao et al. showed that *lncRNA GAS5* was down-regulated in cervical cancer and decreased expression of *lncRNA GAS5* predicts a poor prognosis in cervical cancer patients [26]. Wang et al. observed that over-expression of *lncRNA HOTAIR* promotes tumor growth and metastasis in human osteosarcoma [27]. Besides, Svoboda et al. also observed that over-expression of *lncRNA HOTAIR* levels positively correlated between blood and tumor and it may serve as a potential prognostic marker in CRC [28]. Shi et al. suggested that down-regulated *lncRNA BANCR* promotes the proliferation of colorectal cancer cells via down-regulation of p21 expression [29]. However, the role of *lncRNA Sox2ot* in the carcinogenesis of CRC are still unknown.

Recent studies have reported that *Sox2* participated in cell reprogramming and tumorigenesis of various cancers [30]. *lncRNA Sox2ot* played a role in the processes related to *Sox2* transcription, acting as an enhancer. Askarian-Amiri et al. revealed that ectopic expression of *lncRNA Sox2ot* led to an increase in

Sox2 expression and reduced proliferation of breast cancer cells. They indicated that *lncRNA Sox2ot* played a key role in the progression of breast cancer [31]. Hou et al. showed *lncRNA Sox2ot* was up-regulated in lung cancers, and high *lncRNA Sox2ot* expression was correlated with patients' survival time after surgery [17].

In the present study, we investigated the expression and clinical significance of *lncRNA Sox2ot* in CRC patients. Our results showed that *lncRNA Sox2ot* expression in CRC tissues was significantly higher than that in matched adjacent non-cancerous tissues. The relationships of *lncRNA Sox2ot* with various clinical features of CRC were analyzed, we found that *lncRNA Sox2ot* expression was proven to be associated with N stage, T stage, TNM stage, histological differentiation grade, lymph node involvement and distant metastasis, suggesting that *lncRNA Sox2ot* might be involved in the carcinogenesis of CRC. Furthermore, Kaplan-Meier analysis with the log-rank test indicated that patients with a high level of *lncRNA Sox2ot* expression had significantly shorter overall survival than those with a low level of *lncRNA Sox2ot* expression. In Cox regression analysis, our results suggested that *lncRNA Sox2ot* expression level was independent prognostic factors for overall survival of CRC patients. All the results indicated that high *lncRNA Sox2ot* level was a promising non-invasive bio-marker for prognosis of CRC patients. Moreover the further studies are needed to elucidate the the mechanism of *lncRNA Sox2ot* in CRC.

Conclusion

In conclusion, our data suggested that *lncRNA Sox2ot* up-regulation was associated with aggressive progression and poor prognosis in CRC. *lncRNA Sox2ot* could be used as a new biomarker and a potential therapeutic target for CRC.

Abbreviations

Long non-coding RNA (lncRNA)

colorectal cancer (CRC)

quantitative real-time polymerase chain reaction (qRT-PCR)

overall survival (OS)

cycle threshold (CT)

standard deviation (SD)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Southwest Hospital, Army Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

The subjects provided written informed consent for the publication of any associated data and accompanying images.

Data availability All data generated or analysed during this study are included in this published article.**Funding** No funding was received**Competing interests**The authors declare that they have no competing interests.**Authors' contributions** K.Z. design of the work; T.M., Z.H. the acquisition, analysis, X.W., Y.P. interpretation of data; Y.C., Y.D. the creation of new software used in the work; Z.R., Z.W. have drafted the work or substantively revised it. All authors read and approved the final manuscript.**Acknowledgements** Not applicable.

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Tables

Table 1. Summary of the primers used in the study

Gene	Primer Sequence	Length
Sox2ot	F: 5'-GCTCGTGGCTTAGGAGATTG-3'	239 bp
	R: 5'-CTGGCAAAGCATGAGGAACT-3'	
GAPDH	F: 5'-ACAGTCAGCCGCATCTTCTT-3'	239 bp
	R: 5'-GACAAGCTTCCCGTTCTCAG-3'	

Table 2. The relationship between *lncRNA Sox2ot* expression and clinicopathological characteristic in CRC patients

Parameters	No.	LncRNA Sox2ot expression level		χ^2	<i>P</i> values a
		Low	High		
Age (years)				0.218	0.640
≤50	54	20	34		
>50	63	26	37		
Gender				0.149	0.700
Female	56	21	35		
Male	61	25	36		
Tumor size				0.020	0.887
≤4 cm	55	22	33		
>4 cm	62	24	38		
N stage b				9.205	0.002
N0-N1	51	28	23		
N2-N3	66	18	48		
T stage b				10.580	0.001
T1-T2	57	31	26		
T3-T4	60	15	45		
TNM stage b				7.035	0.008
I and II	51	27	24		
III and IV	66	19	47		
Histological differentiation grade				9.205	0.002
High/moderate	51	28	23		
Low/unknown	66	18	48		
Tumor histology				0.833	0.361
Adenocarcinoma	57	20	37		
Mucinous adenocarcinoma	60	26	34		
Lymph node involvement				7.000	0.008
Absent	56	29	27		
Present	61	17	44		

Distant metastasis				8.260	0.004
Negative	57	30	27		
Positive	60	16	44		
Primary tumor localization				0.085	0.770
Sigmoid/rectum	54	22	32		
Colon	63	24	39		
Karnofsky performance status				0.812	0.368
≤80	55	24	31		
>80	62	22	40		

a Comparisons were made by Pearson Chi-square test.

b Staging according to International Union Against Cancer (UICC).

Statistical analyses were performed by the Pearson χ^2 test.

$P < 0.05$ is considered significant.

Table 3. Univariate and multivariate analysis of prognostic factors using Cox regression model.

Parameters	Univariate analysis		Multivariate analysis	
	HR (95% CI)	<i>P</i> values	HR (95% CI)	<i>P</i> values
<i>LncRNA Sox2ot</i>	3.783 (2.119-6.756)	0.000	3.783 (2.119-6.756)	0.000
Age	1.042 (0.628-1.730)	0.872		
Gender	1.087 (0.653-1.812)	0.748		
Tumor size	0.926 (0.559-1.534)	0.765		
N stage	2.148 (1.251-3.688)	0.006		
T stage	1.808 (1.082-3.019)	0.024		
TNM stage	0.998 (0.603-1.654)	0.995		
Histological differentiation grade	1.537 (0.916-2.577)	0.104		
Tumor histology	0.667 (0.401-1.110)	0.119		
Lymph node involvement	1.757 (1.045-2.953)	0.033		
Primary tumor localization	1.140 (0.688-1.890)	0.611		
Karnofsky performance status	1.551 (0.923-2.606)	0.097		
Distant metastasis	1.778 (1.059-2.985)	0.029		

HR, hazard ratio; CI, confidence interval; $P < 0.05$ was considered statistical significant.

Figures

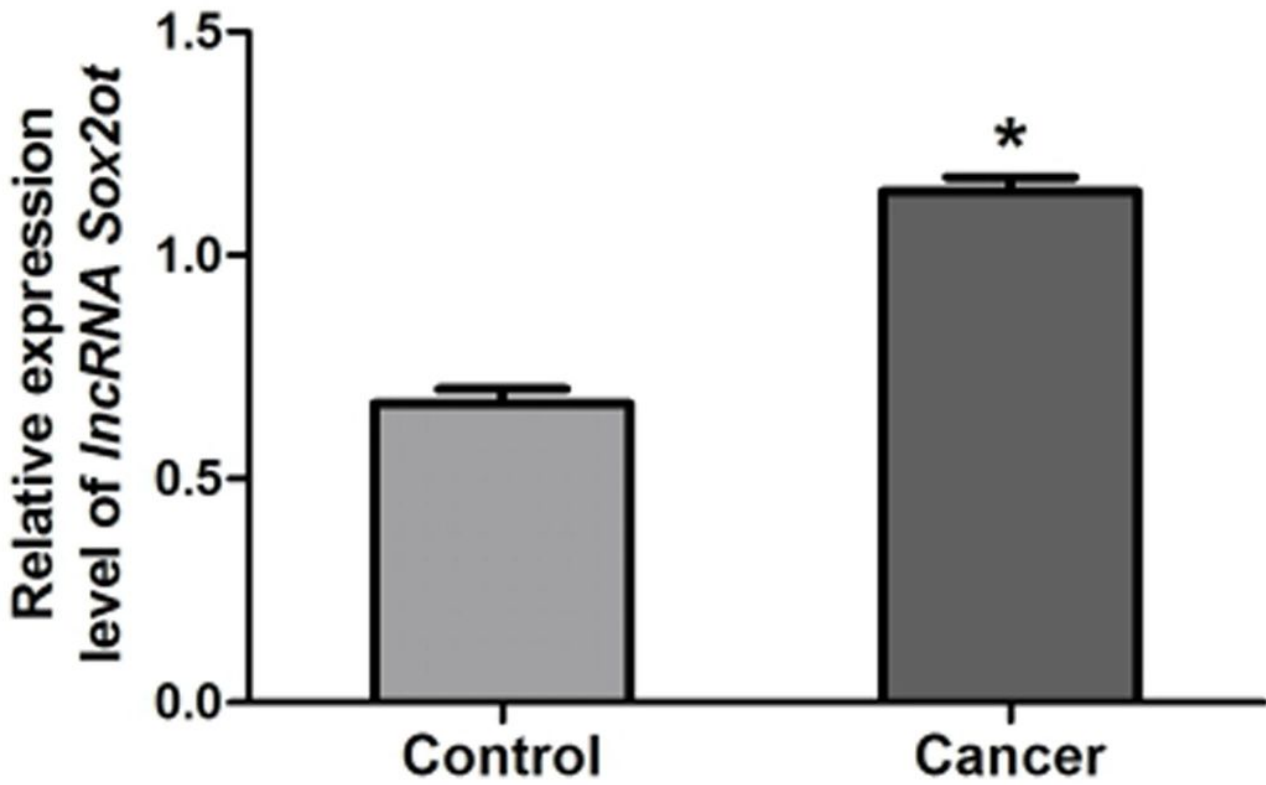


Figure 1

Relative expression of lncRNA Sox2ot in CRC tissues and adjacent non-cancerous tissues measured by qRT-PCR assay. The relative mRNA expression of lncRNA Sox2ot in CRC tissues were significantly higher than that in controls. *, $P < 0.05$.

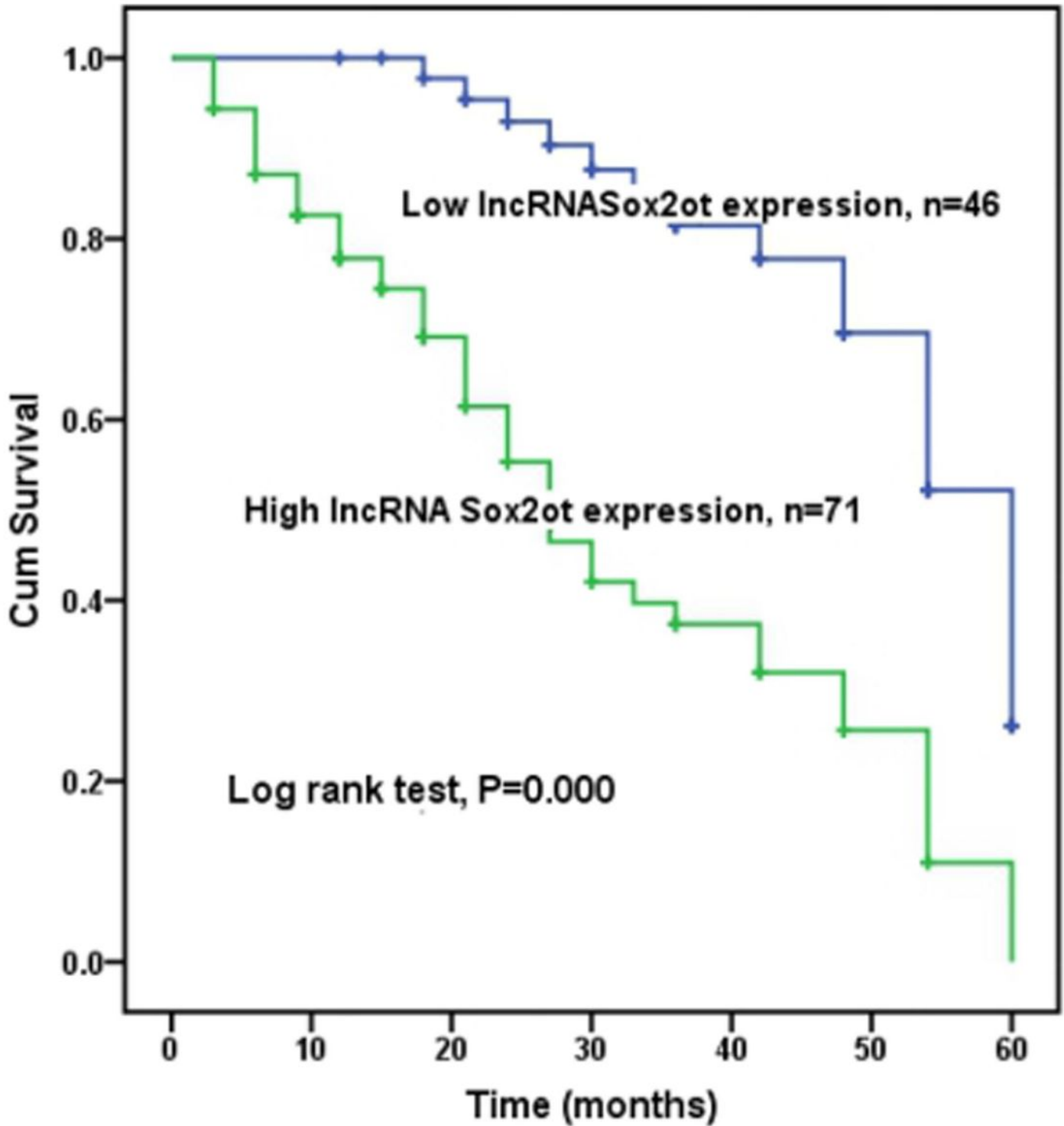


Figure 2

Kaplan-Meier survival curves for CRC patients based on the expression of lncRNA Sox2ot. P value was calculated using the log-rank test. Patients with high lncRNA Sox2ot expression had a shorter survival time than those with low lncRNA Sox2ot expression ($\chi^2 = 25.909, P=0.000$).