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Upregulation of tissue long noncoding RNA X inactive specific transcript predicts poor postoperative survival in patients with non-small cell lung cancer

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Abstract

X inactive specific transcript (XIST) is a novel long noncoding RNA (lncRNA) which has been reported to be frequently upregulated in various human cancer types and to function as an oncogene. It has been reported that the expression of lncRNA XIST was upregulated in non-small cell lung cancer (NSCLC). In the present study, we aimed to investigate the clinical significance and prognostic value of XIST in patients with NSCLC.

A total of 156 pairs of NSCLC and corresponding adjacent normal lung tissue samples were obtained from NSCLC patients who had undergone surgery from July 2014 to March 2019. The Student's t test was used in different treated groups for statistical analysis. The association between XIST expression and clinicopathological features of NSCLC patients was evaluated using the chi-squared test. Survival curves were plotted using Kaplan-Meier method and compared by log-rank test.

The expression of XIST was significantly higher in NSCLC samples compared to non-cancerous samples ($P < .001$). Statistically significant correlations were observed between high tissue XIST expression level and lymph node metastasis ($P = .036$) and high Tumor Node Metastasis (TNM) stage ($P = .002$). The log-rank test indicated that patients with increased XIST expression experienced poor overall survival ($P = .006$). Multivariate Cox regression analysis showed that XIST expression level (hazard ratio = 2.645, 95% confidence interval: 1.672–7.393, $P = .029$) was an independent factors in predicting the overall survival of NSCLC patients.

The present study found that XIST expression level was significantly associated with advanced pathological stage and high TNM stage in NSCLC. Furthermore, upregulation of tissue lncRNA XIST predicts poor postoperative survival in patients with NSCLC.

Abbreviations: 95%CI = 95% confidence interval, HR = hazard ratio, lncRNAs = long noncoding RNAs, NSCLC = non-small cell lung cancer, OSCC = ovarian squamous cell carcinoma, qPCR = Quantitative real-time RT-PCR analysis, SCLC = small cell lung cancer, XIST = X inactive specific transcript.

Keywords: clinical significance, long noncoding RNAs, lung cancer, non-small cell lung cancer, prognosis, X inactive specific transcript

1. Introduction

Lung cancer ranks the most common cancer all over the world and its most common type is non-small cell lung cancer (NSCLC).[1] According to WHO classification, lung adenocarcinoma (LUAD) and lung squamous cell cancer (LSCC) as two major histological types of NSCLC 4, and LUAD has become the most frequent histological type of NSCLC.[2] Although great progress in diagnostic and therapeutic methods has been made, the prognosis of NSCLC patients after surgery remains dismal.[3–5] Therefore, it is essential to explore new targets for NSCLC diagnosis and treatment.

Long noncoding RNAs (lncRNAs) stand for a class of transcribed RNA molecules with length of more than 200 nucleotides.[6] LncRNAs encode no protein products but regulate gene expression at multiple levels. LncRNAs are found to be involved in a diverse aspect of the biology, such as gene expression regulation, chromatin structure, epigenetic control, and splicing.[7,8] In addition, aberrant levels of lncRNAs can also play a key role in the pathological progression of many diseases, including a variety of human cancers.[9,10]

In recent years, the lncRNA X inactive specific transcript (XIST) has been reported to be frequently upregulated in certain

common types of cancer, and its upregulation is associated with tumour progression and poor prognosis of cancer patients, including cervical cancer, bladder cancer, pancreatic cancer, colorectal cancer, ovarian cancer, gastric cancer, renal cell carcinoma, hepatocellular carcinoma, and oesophageal squamous cell carcinoma.[11–19]

The expression level XIST has been found to be up-regulated in NSCLC, and downregulation of XIST inhibited cell proliferation, migration, invasion and EMT of NSCLC.[20–24] In the present study, we aimed to investigate the clinical significance and prognostic value of XIST in patients with NSCLC.

2. Materials and methods

2.1. Patients and tissue specimens

A total of 156 pairs of NSCLC and corresponding adjacent normal lung tissue samples were obtained from 156 NSCLC patients who had undergone surgery at the Department of Cardiothoracic surgery, Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine from July 2014 to March 2019. None of these patients had received chemotherapy or radiotherapy before surgery. The collected tissue samples were confirmed by histopathological examination, immediately snap frozen in liquid nitrogen and stored at −80°C until required. This study was approved by the Ethical Committee of Ruikang Hospital Affiliated to Guangxi University of Chinese Medicine. Clinical data was obtained from medical record of patients. The clinicopathological characteristics of these patients were listed in Table 1.

2.2. Quantitative real-time RT-PCR analysis (qPCR)

Total RNA was extracted using TRIzol (Invitrogen, USA). cDNA was synthesized using the PrimeScript RT reagent Kit (TaKaRa), and qPCR was performed using the Real-Time Quantitative PCR

SYBR Green kit (TaKaRa) on the ABI 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA). PCR reactionconditions were: 55 seconds at 95°C, and then 12 seconds at 95°C and 32 seconds at 58.5°C for 40 cycles. The expression of XIST was normalized to GAPDH using $2^{-\Delta\Delta CT}$ method. Sequences of primers used in PCR reactions were: XIST, forward: 5′-ACGCTGCATGTGTCCTTAG-3, reverse: 5′-GAGCCTCTTATAGCTGTTTG-3′; GAPDH, forward: 5′-GGAATCCACTGGCGTCTTCA-3′, reverse: 5′-GGTTCACGCC-CATCACAAAC-3′.

2.3. Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA) and SPSS statistical software, version 19.0 (SPSS Inc., Chicago, IL). The Student's t test was used in different treated groups for statistical analysis. The association between XIST expression and clinicopathological features of NSCLC patients was evaluated using the chi-squared test. Survival curves were plotted using Kaplan-Meier method and compared by log-rank test. $P < .05$ was considered to indicate a statistically significant difference.

3. Results

3.1. The expression level of XIST in NSCLC tissues and adjacent normal tissues

We assessed XIST expression in 156 NSCLC samples and 156 paired non-cancerous samples. The expression of XIST was significantly higher in NSCLC samples compared to non-cancerous samples ($P < .0001$, shown in Fig. 1). We divided 156 NSCLC patients into two groups according to the levels of XIST expression level. The cut-off point was the median expression level of XIST in NSCLC samples (high expression group, n = 79; low expression group, n = 77).

3.2. The correlation between XIST expression level and clinicopathological features of NSCLC patients

We then investigated the associations of tissue XIST expression level with various clinicopathological characteristics of human NSCLC. Statistically significant correlations were observed

Table 1

Relationship between the levels of XIST and clinicopathological variables in NSCLC patients.

| Variables	Cases(n)	XIST expression High(n = 79)	XIST expression Low(n = 77)	P value
Gender				
Male	83	43	40	.873
Female	73	36	37	
Age (yr)				
< 60	68	28	40	.052
≥60	88	51	37	
Smoking history				
Positive	65	35	30	.52
Negative	91	44	47	
Family history of lung cancer				
No	75	34	41	.262
Yes	81	45	36	
Tumor size (cm)				
<3	68	30	38	.196
≥3	88	49	39	
Lymph Node Metastasis				
Negative	69	28	41	.036
Positive	87	51	36	
TNM stage				
I/II	71	26	45	.002
III	85	53	32	
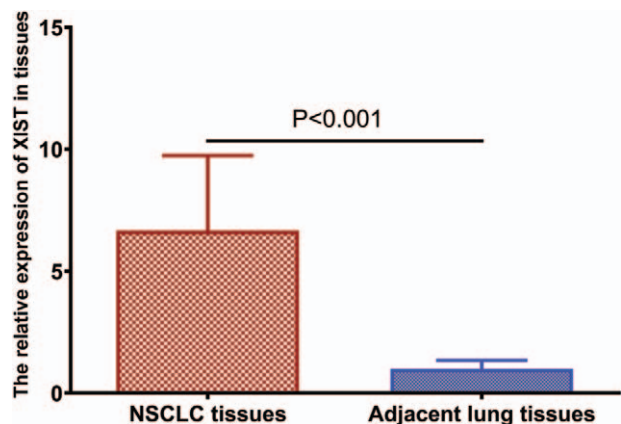
Figure 1. Relative levels of XIST in NSCLC tissues and adjacent non-tumor tissues were determined by quantitative RT-PCR (qRT-PCR).
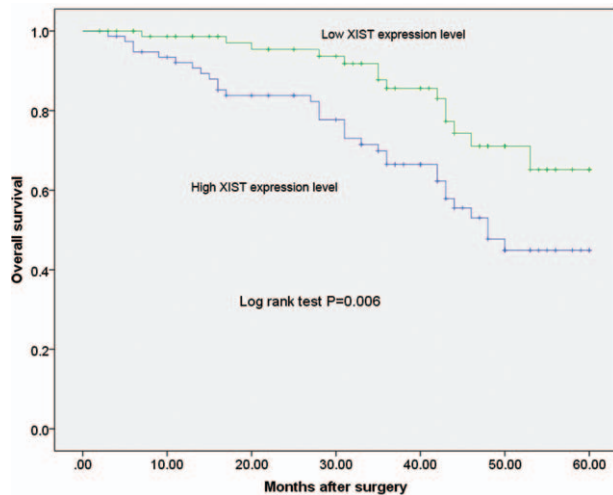
Figure 2. Prognostic value of XIST for NSCLC patients was analyzed by Kaplan-Meier analysis.

between high tissue XIST expression level and lymph node metastasis (P = .036) and high TNM stage (P = .002; shown in Table 1). However, there was no significant association between XIST expression level and other clinicopathological parameters, including age, gender, tumor size, smoking history, and family history of lung cancer (all P > .05; shown in Table 1).

3.3. Increased XIST expression level predicts the poor prognosis of NSCLC patients

Using kaplan-Meier survival plots and log-rank analyses, we evaluated the association of XIST expression with overall survival. The log-rank test indicated that patients with increased XIST expression experienced poor overall survival (P = .006, shown in Figure 2). To determine the possibility of XIST as an independent risk factor for poor prognosis, both clinicopathological factors and the level of XIST expression were evaluated by multivariate Cox regression analysis. Results showed that XIST expression level (HR = 2.645, 95%CI: 1.672–7.393, P = .029) was an independent factors in predicting the overall survival of NSCLC patients (shown in Table 2).

4. Discussion

NSCLC is one of the most common malignancies around the globe. The pathogenesis of NSCLC is a multistep and multistage

Table 2

Multivariate analysis of overall survival in patients with NSCLC.

Variable	Hazard ratio	95% CI	P value
Gender	0.784	0.563–1.357	.583
Age	1.749	0.722–3.855	.129
Smoking history	1.937	0.751–3.559	.564
Family history of lung cancer	2.744	0.821–5.682	.143
Tumor size	1.693	0.832–4.229	.088
Lymph Node Metastasis	3.672	2.832–11.943	.009
TNM stage	3.829	2.553–14.893	.005
XIST expression level	2.645	1.672–7.393	.029

process involving environmental factors and genetic factors. Despite numerous years of basic and clinical research on NSCLC, the 5-year survival rate still remains less than 15%.[25,26] Therefore, exploring the molecular mechanisms and searching for key molecules during initiation and progression of NSCLC are important for the development of novel treatment.

Long non-coding RNAs, RNA transcripts longer than 200 bp in length, are the major part of transcribed noncoding RNA.[27] Previous studies demonstrated that lncRNAs are essential players in the pathogenesis of human diseases, particularly different types of human cancer.[28,29] Therefore, lncRNAs may be promising therapeutic targets for the treatment of cancer.

XIST is an lncRNA required for transcriptional silencing of the X chromosome, which plays an important role in the inactivation of the X chromosome. In recent years, increasing evidence has shown that XIST is closely related to the occurrence and progression of many human cancers. For example, Zhang et al found that lncRNA XIST was found to act as a miR-497–5p sponge and to regulate the level of PDCD4, which is targeted by miR-497-5p. lncRNA XIST was observed to be downregulated in the HCC tissues and positively correlated with the expression of PDCD4, revealing that the XIST/miR-497-5p/PDCD4 axis participates in HCC development and that XIST could be used as a biomarker of HCC.[18] Chen et al found that the effects of XIST/miR-140-5p/ORC1 axis on the progression of cervical cancer which will shed new light on epigenetic diagnostics and therapeutics in cervical cancer.[11] Sun et al found that XIST was down-regulated in RCC tissues and cells. Overexpression of XIST significantly suppressed cell proliferation and induced cell G0/G1 arrest in vitro and inhibited tumor growth in vivo. They further found that XIST could directly interact with miR-106b-5p and increase the expression of P21. Thus, XIST positively regulated the expression of P21 through sponging miR-106b-5p, and played a tumor suppressor role in RCC. Moreover, they found that curcumin could regulate XIST/miR-106b-5p/P21 axis in RCC cells.[17] Chen et al found that lncRNA XIST was significantly up-regulated in gastric cancer tissues and cell lines. Overexpression of lncRNA XIST was markedly associated with larger tumor size, lymph node invasion, distant metastasis and TNM stage in gastric cancer patients. Functionally, knockdown of lncRNA XIST exerted tumor-suppressive effects by inhibiting cell proliferation, migration and invasion in vitro and tumor growth and metastasis in vivo. Furthermore, an inverse relationship between lncRNA XIST and miR-101 was found. Polycomb group protein enhancer of zeste homolog 2 (EZH2), a direct target of miR-101, could mediated the biological effects that lncRNA XIST exerted.[30] Zuo et al found that XIST was up-regulated in epithelial ovarian cancer (EOC) tissues and cell lines. The expression of XIST was closely related to the tumor grade, distant metastasis, and FIGO stage in the EOC patients. The Cox regression analysis showed that high XIST expression was an independent predictor of prognosis in patients with EOC. In in vitro experiments, reducing XIST expression significantly suppressed cell proliferation, migration and invasion in EOC cells.[19] Shen et al found that XIST was frequently upregulated while miR-429 was commonly downregulated in pancreatic cancer tissues, especially in metastatic pancreatic cancer tissues. Knockdown of XIST in two pancreatic cancer cell lines caused inhibition of migration, invasion and EMT capacities. Forced expression of miR-429 exerted the similar tumor suppressing effects. XIST repressed miR-429 expression thus upregulated ZEB1, one of the targets of miR-429. ZEB1 mediated the tumor

suppressing roles of XIST knockdown in pancreatic cancer cells. They identified the critical axis of XIST/miR-429/ZEB1 in pancreatic cancer cell migration, invasion and EMT, which may aid in developing new therapeutic strategies for pancreatic cancer.[16]

The expression level XIST has been found to be up-regulated in NSCLC, and downregulation of XIST inhibited cell proliferation, migration, invasion and EMT of NSCLC. Furthermore, upregulation of lncRNA-XIST was associated with cisplatin resistance in NSCLC by downregulating miRNA-144-3p in H460/DDP and A549/DDP cells, a murine A549/DDP tumor xenograft, and human tumor tissues from patients with cisplatin-resistant NSCLC.[20] In the present study, we aimed to investigate the clinical significance and prognostic value of XIST in patients with NSCLC. We assessed XIST expression in 156 NSCLC samples and 156 paired non-cancerous samples. The expression of XIST was significantly higher in NSCLC samples compared to non-cancerous samples. We then investigated the associations of tissue XIST expression level with various clinicopathological characteristics of human NSCLC. Statistically significant correlations were observed between high tissue XIST expression level and lymph node metastasis and high TNM stage. However, there was no significant association between XIST expression level and other clinicopathological parameters, including age, gender, tumor size, smoking history, and family history of lung cancer. Using kaplan-Meier survival plots and log-rank analyses, we evaluated the association of XIST expression with overall survival. The log-rank test indicated that patients with increased XIST expression experienced poor overall survival. To determine the possibility of XIST as an independent risk factor for poor prognosis, both clinicopathological factors and the level of XIST expression were evaluated by multivariate Cox regression analysis. Results showed that XIST expression level was an independent factors in predicting the overall survival of NSCLC patients.

In conclusions, the present study found that XIST expression level was significantly associated with advanced pathological stage and high TNM stage in NSCLC. Furthermore, upregulation of tissue lncRNA XIST predicts poor postoperative survival in patients with NSCLC.

Author contributions

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