Original Article

Downregulation of lncRNA TUG1 is involved in ankylosing spondylitis and is related to disease activity and course of treatment

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Summary   **Long non-coding RNA taurine-upregulated gene 1 (lncRNA TUG1) promotes osteosarcoma, while its involvement in other bone diseases, such as ankylosing spondylitis (AS) is unknown. Expression of TUG1 in serum and open sacroiliac biopsies of AS patents and healthy controls was detected by real-time quantitative PCR (qRT-PCR). Ankylosing spondylitis disease activity score (ASDAS) system was used to evaluate disease activity. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic value of lncRNA TUG1 for AS. Chi-square test was performed to analyze the correlations between TUG1 expression and patients' clinicopathological data. Patients were divided into 2 groups (high and low expression groups) according to the median expression level of TUG1 and were followed-up for 5 years after discharge. Treatment courses and rehospitalization rate were compared between two groups. It was observed that TUG1 expression level was significantly lower in AS patients than in healthy controls in both serum and biopsies. Reduced expression level of TUG1 distinguished AS patients from controls. LncRNA TUG1 expression was significantly correlated with patients' smoking habits, disease activity, and course of disease. Patients in high expression group showed longer hospitalization time and higher rehospitalization rate. We therefore conclude that expression of lncRNA TUG1 was inhibited in AS patients and downregulation of lncRNA TUG1 is related to higher disease activity, longer course of treatment and higher rehospitalization rate.**

Keywords: lncRNA TUG1, ankylosing spondylitis

1. Introduction

As a type of immune-mediated chronic disease characterized by new bone formation in the axial skeleton and inflammatory responses (*1*), ankylosing spondylitis (AS) causes deterioration of functioning, irreversible structural damage and disability, seriously affecting patients' quality of life (*2*). Different treatment strategies have been developed to treat AS, however most treatment therapies failed to achieve satisfactory outcomes. Treatment with nonsteroidal anti-inflammatory drugs delays disease progression but is not sufficient to control disease symptoms (*3*). Anti–tumor necrosis factor (TNF) therapy now has been widely used in the treatment of active patients, but unacceptable side effects were observed in some patients (*4,5*). Treatment of AS is mainly challenged by the unclear pathogenesis (*6*), and identification of novel molecular treatment targets is always needed.

Onset, development and progression of AS is a complex process with multiple internal and environment factors involved, and genetic factors play a central role in this disease (*7,8*). A recent study has shown that the development of AS is also accompanied by changes in expression pattern of a large set of long non-coding RNAs (lncRNAs) (*9*), which is a subgroup of non-coding RNAs that play critical roles in human diseases (*10*). However, the roles of most lncRNAs in AS

remain unclear. LncRNA taurine-upregulated gene 1 (TUG1) has been proved to be an oncogenic lncRNA in osteosarcoma (*11*). In our study we observed that TUG1 was downregulated in AS and is correlated with course of treatment and re-hospitalization.

2. Materials and Methods

2.1. *Specimens*

Clinical data of 82 patients with AS were retrospectively reviewed. Those patients were diagnosed and treated in The Third Affiliated Hospital of Nanchang University from January 2010 to January 2012. Inclusion criteria: 1) patients diagnosed as AS and treatment for the first time; 2) patients completed treatment; 3) patients completed follow-up and have complete follow-up data; 4) patients without other severe diseases and bone disease; 5) patients and/or their families willing to participate. Exclusion criteria: 1) patients treated before admission; 2) patients transferred to other hospitals during treatment; 3) patient died during treatment or follow-up. Those patients included 44 males and 38 females, and age ranged from 12 to 44 years, with a mean age of 27.1 ± 4.4 years. Serum samples of all AS patients and open sacroiliac biopsies of 34 patients were obtained from specimen library of The Third Affiliated Hospital of Nanchang University. Besides that, our study also included 32 controls and AS were excluded from those patients through open sacroiliac biopsies. Serum samples and open sacroiliac biopsies of those controls were also obtained from specimen library of The Third Affiliated Hospital of Nanchang University. Control group included 18 males and 14 females, and age ranged from 14 to 46 years, with a mean age of 28.7 ± 5.1 years. No significant differences in age, gender and other basic clinical data were found between the two groups. This study was approved by the ethics committee of The Third Affiliated Hospital of Nanchang University and all participants and/or their families signed informed consent.

2.2. *Disease activity evaluation*

Ankylosing spondylitis disease activity score (ASDAS) (*12*) was used to evaluate disease activity. The criteria were: inactive disease, < 1.3; moderate disease activity, between 1.3 and 2.1; high disease activity, between 2.1 and 3.5; very high disease activity, > 3.5.

2.3. *Real-time quantitative PCR (qRT-PCR)*

Trizol reagent (Invitrogen, USA) was used to extract total RNA from biopsies and serum. Biopsies were ground in liquid nitrogen before the addition of Trizol reagent to achieve complete cell lysis. Reverse transcription was performed (55°C for 15 min and 85°C for 15 min) using SuperScript IV Reverse Transcriptase (Thermo Fisher Scientific) to synthesize cDNA, followed by PCR reaction using SYBR® Green Real-Time PCR Master Mixes. Sequences of primers used in PCR reactions were: 5'-CTGAAGAAAGGCAACATC-3' (sense) and 5'-GTAGGCTACTACAGGATTTG-3' (antisense) for TUG1; 5'-GACCTCTATGCCAACACAGT-3' (forward) and 5'-AGTACTTGCGCTCAGGAGGA-3' (reverse) for human β-actin. Reaction conditions of PCR: 50 s at 95°C, followed by 40 cycles of 15 s at 95°C and 40 s at 58°C. Ct values were processed using $2^{-\Delta\Delta CT}$ method, and TUG1 expression was normalized to β-actin endogenous control.

2.4. *Enzyme-Linked ImmunoSorbent Assay (ELISA)*

Serum levels of C-reactive protein (CRP) were measured using a human CRP quantikine ELISA Kit (DCRP00, R&D Systems). All operations were performed in strict accordance with manufacturer's instructions. Serum levels of CRP were normalized to mg/L.

2.5. *Statistical analysis*

All data analyses were performed using Graphpad Prism 6 software. TUG1 expression data were recorded as ($\overline{\chi} \pm$ sem) and compared by unpaired *t* test (between 2 groups) and one way analysis of variance followed by least significant difference (LSD) test (among multiple groups). Chi-square test was used for comparisons of count data. Correlation analyses were performed by Pearson correlation analysis. *P* < 0.05 was considered to be statistically significant.

3. Results

3.1. *Comparison of TUG1 expression in serum and open sacroiliac biopsies between AS patients and controls*

Differential expression in patients and healthy people usually indicates the involvement of a gene in a certain disease. Therefore, we first detected the expression of TUG1 in serum and open sacroiliac biopsies of both AS patients and controls. Results showed that TUG1 expression was significantly downregulated in AS patients compared to healthy controls in both serum (Figure 1a) and open sacroiliac biopsies (Figure 1b). Therefore, downregulation of TUG1 is likely involved in the pathogenesis of AS.

3.2. *Evaluation of diagnostic values of TUG1 expression for AS*

Biomarkers have shown potential in assisting disease diagnosis. TUG1 expression was detected in both serum and open sacroiliac biopsies and differential expression
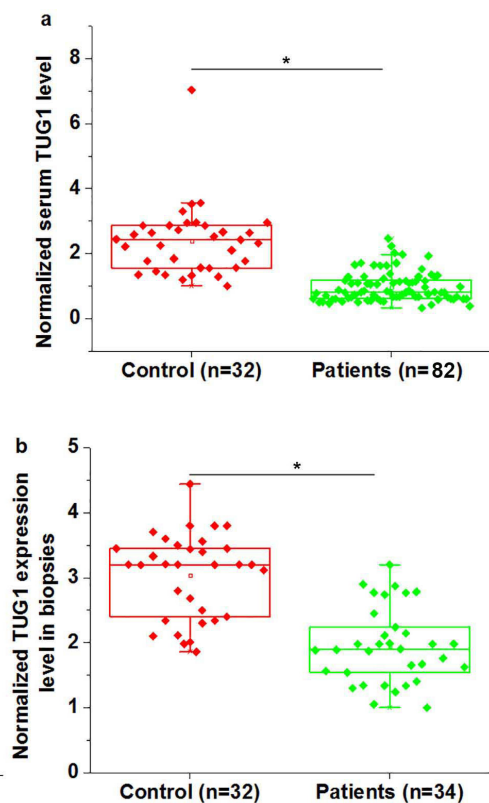
Figure 1. Comparison of TUG1 expression in serum and open sacroiliac biopsies between AS patients and controls. Normalized expression levels of lncRNA TUG1 in serum **(a)** and open sacroiliac biopsies **(b)** of AS patients and controls. Notes: *, $p < 0.05$.
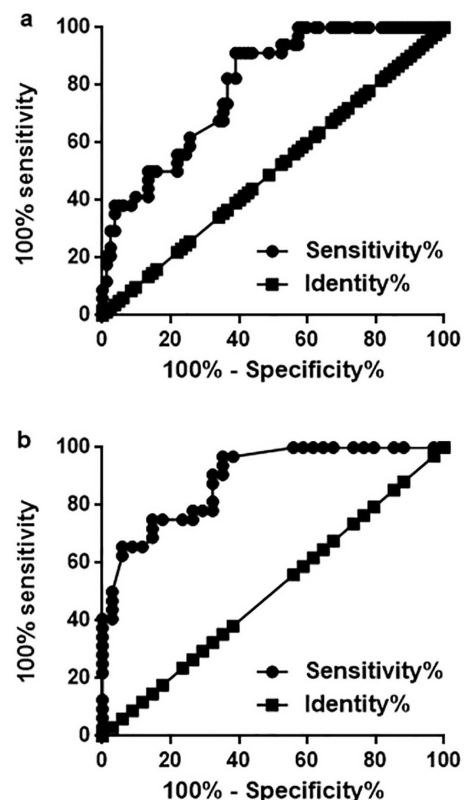

Figure 2. Evaluation of diagnostic values of TUG1 expression for AS. The ROC curve of the use of TUG1 expression in serum **(a)** and biopsies **(b)** for the diagnosis of AS.

was observed between AS patients and healthy controls. Therefore, ROC curve analysis was performed to evaluate the diagnostic values of TUG1 expression for AS. As shown in Figure 2a, area under the curve (AUC) of TUG1 expression in serum was 0.7968 with standard error of 0.04158 and 95% confidence interval of 0.7153 to 0.8783 ($p < 0.0001$). In addition, AUC of TUG1 expression in biopsies for the diagnosis of AS was 0.8911 with standard error of 0.03767 and 95% confidence interval of 0.8172 to 0.9649 ($p < 0.0001$).

3.3. *Serum levels of TUG1 were negative correlated with serum levels of CRP in ankylosing spondylitis patients*

Pearson correlation analysis revealed that serum levels of TUG1 were negative correlated with serum levels of CRP in ankylosing spondylitis patients ($r = -0.8091$, $R^2 = 0.6431$, $p < 0.0001$) (Figure 3).

3.4. *Serum levels of TUG1 in patients with different disease activities*

According to the ASDAS system, there were 22 cases of inactive disease (ID), 18 cases of moderate disease activity (MD), 28 cases of high disease activity (HD) and 14 cases of very high disease activity (VHD). As
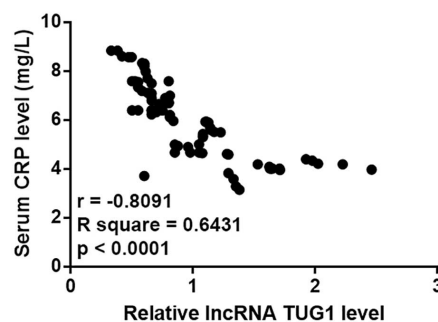

Figure 3. Pearson correlation analysis of the correlation between serum levels of TUG1 and CRP in ankylosing spondylitis patients.

shown in Figure 4, serum levels of TUG1 decreased significantly with increase of degree of disease activity ($p < 0.05$).

3.5. *Correlations between TUG1 expression and patients' clinicopathological data*

Patients were divided into high and low expression groups according to the median expression level of TUG1. Chi square test was performed to investigate the correlations between TUG1 expression and patients' clinicopathological data. As shown in Table 1 and 2, TUG1 expression was significantly correlated with

Table 1. Correlations between serum levels of TUG1 and patients' clinicopathological data

Items	Groups	Cases	High-expression	Low-expression	χ^2	*p* value
Gender	Male	44	20	24	0.78	0.37
	Female	38	21	17		
Age	> 25 (years)	46	21	25	0.79	0.37
	< 25 (years)	36	20	16		
Course of disease	> 5 years	40	13	27	9.57	0.002
	< 5 years	42	28	14		
Smoking	Yes	37	23	14	3.99	0.046
	No	45	18	27		
Drinking	Yes	51	24	27	0.03	0.87
	No	31	17	14		

Table 2. Correlations between TUG1 expression in biopsies and patients' clinicopathological data

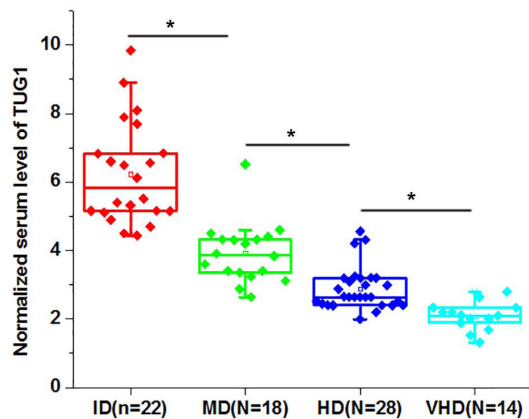| Items	Groups	Cases	High-expression	Low-expression	χ^2	*p* value
Gender	Male	20	9	11	0.49	0.49
	Female	14	8	6		
Age	> 25 (years)	18	10	8	0.47	0.49
	< 25 (years)	16	7	9		
Course of disease	> 5 years	19	5	14	9.66	0.002
	< 5 years	15	12	3		
Smoking	Yes	18	12	6	4.25	0.04
	No	16	5	11		
Drinking	Yes	21	9	12	1.12	0.29
	No	13	8	5		

Figure 4. Serum levels of TUG1 in patients with different disease activities. Notes:*, $p < 0.05$.
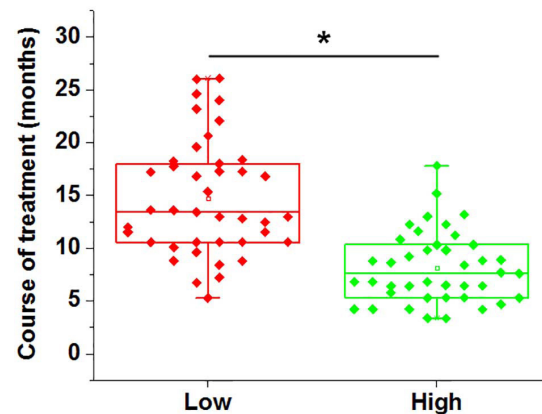

Figure 5. Comparison of course of treatment between high and low serum TUG1 groups. Notes:*, $p < 0.05$.

patients' course of disease and smoking habit ($p < 0.05$), but not gender, age as well as drinking habit ($p > 0.05$).

3.6. Comparison of course of treatment and re-hospitalization rate between high and low serum TUG1 groups

As shown in Figure 5, course of treatment is significantly longer in low expression group than in high expression group. During follow-up, re-hospitalization occurred in 18 cases of low expression group, accounting for 43.9%. In contrast, re-hospitalization only occurred in 8 cases of high expression group, accounting for 19.5%. Therefore, TUG1 is likely related to course of treatment and re-

hospitalization of AS patients.

4. Discussion

The key finding of our study is that as a lcnRNA with critical roles in human malignancies such as osteosarcoma (*11*), lncRNA TUG1 is also very likely involved in the pathogenesis of AS. Low expression of TUG1 may delay patients' recovery and increase the risk of re-hospitalization.

LncRNA plays pivotal roles in different types of human diseases (*10*). However, functionality of most lncRNAs in AS still has been characterized. A recent study has shown that levels of circulating lncRNA-

AK001085 were significantly lower in ankylosing spondylitis patients than in healthy controls (*13*), indicating the involvement of this lncRNA in AS. Osteoblastic differentiation plays pivotal roles in the development of AS (*13*). It has been shown that lncRNA TUG1 promotes osteoblast differentiation through miR-204-5p (*14*). Therefore, TUG1 is also likely involved in AS. In our study, significantly downregulated expression of lncRNA TUG1 was observed in AS patients compared to controls in both serum and open sacroiliac biopsies, suggesting that downregulation of TUG1 is involved in AS.

Development of human disease is usually accompanied by changes in certain substances in blood, and monitoring the content of those substances in blood may provide guidance for treatment of disease (*15*). In this study, TUG1 was detected in both serum and biopsies of all AS and controls. ROC curve analysis showed that low expression levels of TUG1 in serum and biopsies effectively distinguish AS patients from healthy controls. Compared with serum TUG1, the diagnostic efficacy of TUG1 expression in biopsies was much higher than that of serum TUG1. However, application of open sacroiliac biopsies is sometimes challenged by its invasive nature. Therefore, measuring serum levels of TUG1 may be used to assist the diagnosis of AS and cases of open sacroiliac biopsy is not applicable. Expression of lncRNAs is affected by many factors including aging (*16*), alcohol abuse (*17*) and tobacco consumption (*18*). In our study, expression of TUG1 showed no significant correlations with patients' age, gender or drinking habits. However, a significant correlation was observed between patients' smoking habit and expression of TUG1 in both serum and biopsies. Therefore, patients' smoking habit should be taken into consideration in the use of TUG1 for the diagnosis of AS.

Disease activity of AS determines its progression rate and symptoms (*19*). Serum levels of TUG1 were found to be significantly increased with increase of disease activities. The long treatment course and high postoperative recurrence rate bring heavy economic and mental burden on patients and their families (*20,21*). In our study, low serum levels of TUG1 were found to be significantly correlated with delayed recovery and increased re-hospitalization rate. Therefore, TUG1 may serve as a potential therapeutic target to inhibit the activity of AS, promote patients' recovery and reduce the risk of recurrence.

Our study is limited by its small sample size due to limited resources. Future studies with bigger sample sizes are needed to further confirm our conclusions. Mechanism of the action of TUG1 in AS is still unknown. Our further direction will focus on the molecular mechanism underlying the role of TUG1 in this disease.

In conclusion, downregulation of TUG1 is very likely involved in the pathogenesis of AS and inhibited TUG1 expression delays patients' recovery and increases recurrence rate.

Authors' contributions

X.L, H. M, and Z.Z. designed and carried out the study. X.L, H.M, Z.Z, D.Y, J.M, and F.C. participated in experiments and statistical analysis. X.L. and J.L. wrote the manuscript. L.J. revised the manuscript. All authors read and approved the final manuscript.

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