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Original Article

Predictive value of lncRNA ZFAS1 in patients with lumbar disc degeneration

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Abstract: Background: Increasing evidences have indicated the association of non-coding RNAs with the progression of lumbar disc degeneration (LDD), but the role of lncRNA ZFAS1 in LDD remains undefined. Therefore, this study was designed to determine the predictive value of lncRNA ZFAS1 in patients with LDD. Methods: A total of 80 patients with LDD confirmed and treated in the Gansu Provincial Hospital from May 2018 to May 2020 were enrolled into the patient group, and 50 healthy controls who concurrently underwent physical examination in our hospital were enrolled into the control group. The expression and diagnostic value of serum lncRNA ZFAS1 in the two groups were determined. The expression of lncRNA ZFAS1 was compared between the two groups before and one month after therapy, and the associations of lncRNA ZFAS1 with inflammatory factors were analyzed. In addition, logistic regression was carried out to analyze risk factors for the prognosis of patients, and corresponding curves about prediction were drawn. Results: The patient group showed a notably higher lncRNA ZFAS1 level than the control group ($P<0.001$), and the area under the receiver operating characteristic (ROC) of lncRNA ZFAS1 in diagnosing LDD was 0.807. In addition, after therapy, the patient group showed a remarkable decrease in serum lncRNA ZFAS1 ($P<0.01$). Serum lncRNA ZFAS1 was positively correlated with serum TNF-α, IL-6 and IL-1β in patients (all $P<0.05$). Moreover, serum lncRNA ZFAS1 in the good efficacy group was notably lower than that in the general efficacy group ($P<0.01$). Age and lncRNA ZFAS1 expression before therapy were independent risk factors for patients' prognosis (both $P<0.05$). Conclusion: LncRNA ZFAS1 is highly expressed in patients with LDD and is a potential prognostic indicator for LDD.

Keywords: LncRNA ZFAS1, lumbar disc degeneration, diagnosis, prediction

Introduction

Lumbar disc herniation is a syndrome induced by the degeneration or accumulated damage of the lumbar disc annulus, which can lead to the protrusion of the nucleus pulposus to the posterolateral or posterior side, compressing or stimulating the spinal nerve and cauda equina [1, 2]. Lumbar disc degeneration (LDD) is a syndrome usually accompanied by severe low back pain [3]. As a common and frequently occurring disease in the department of spinal surgery, it enormously and adversely affects patients' daily life and work [4]. As LDD progresses, patients will suffer inflammatory nerve root edema, nutritional disorders and conduction function damage [5]. Patients may also experience sensory and motor disorders that seriously reduce the quality of life [6]. At the current stage, LDD is mainly treated by conservative treatment and surgical treatment, without unified treatment schemes at home and abroad [7]. Therefore, it is imperative to have a deep and comprehensive understanding of the mechanism of LDD to offer more effective choices for its clinical treatment.

Long-chain non-coding RNAs (lncRNAs) are transcription factors over 200 nucleotides in length, which are considered as waste products produced by metabolism due to the absence of protein coding ability [8]. A growing number of evidences reveal that lncRNAs are associated with various diseases such as cancer, inflammatory diseases and degenerative diseases [9, 10]. Recent studies have demonstrated the

Table 1. Primer sequences

Gene		
ZFAS1	Upstream primers (5'-3')	AAGCCACGTGCACACATCTA
	Downstream primers (5'-3')	CTACTTCCAACACCCGCATT
GAPDH	Upstream primers (5'-3')	TGACCACAGTCCATGCCATCAC
	Downstream primers (5'-3')	GCCTGCTTCACCACCTTCTTGA

relationship between lncRNA imbalance and LDD progression. For instance, Tang et al. [11] revealed that lncRNA TUG1 can induce intervertebral disc degeneration and nucleus pulposus cell apoptosis via the miR-26a/HMGB1 axis and NF-kappaB activation regulation. As a newly discovered lncRNA, lncRNA zinc finger antisense 1 (ZFAS1) is usually studied as carcinogen lncRNA in various cancers [12, 13]. It has been reported to accelerate the migration and invasion of fibroblast-like synovial cells (FLS) and increase C-reactive protein levels in patients with rheumatoid arthritis (RA) [14]. According to these studies, lncRNA ZFAS1 may be involved in the development of LDD as a pro-inflammatory gene. However, its clinical value in LDD is still under investigation.

Therefore, for the first time, this study analyzed the expression and diagnostic value of lncRNAZFAS1 in LDD patients, aiming to provide potential biomarkers for clinical diagnosis and efficacy evaluation.

Methods and materials

Clinical data

A total of 80 patients with LDD confirmed and treated in the Gansu Provincial Hospital during May 2018 and May 2020 were enrolled into the patient group, and 50 healthy controls who underwent physical examination during the same period in our hospital were enrolled into the control group. The imaging and serological indices of patients in the control group were normal. This study was retrospectively conducted with permission from the Ethics Committee of the Hospital (Ethical Approval No.: 2021-165).

Inclusion and exclusion criteria

Inclusion criteria for the patients: Patients with symptoms such as soreness, pain and numbness of lower limbs; patients confirmed with protrusion of intervertebral disc (central spinal canal stenosis, lateral recess stenosis, intervertebral foramen stenosis, and degenerative slip) according to CT, MR and other imaging technologies; patients whose imaging findings were consistent with clinical symptoms; patients who had not received targeted therapy before this study, and; patients with detailed clinical data.

Exclusion criteria of the patients: Patients with comorbid tumor; patients who were unsuitable for surgical therapy; patients with pathological pain, or those who had lumbar surgery before the study.

Treatment methods

In this study, all patients received posterior lumbar interbody fusion based on cervical intervertebral foramen. Specifically, the intervertebral space of the diseased segment was located in each patient in a prone position with the abdomen suspended after general anesthesia. Then, with the diseased segment as the midpoint, a posterior median longitudinal incision about 5 cm long was made, and then the skin, subcutaneous tissue and muscle fascia of the patient were cut separately to expose the transverse process. With the C-arm fluoroscopy, 4 pedicle screws were implanted in bipedicular site and connected with screw connecting rods. The inferior articular process and part of the upper articular process on the affected side were occluded, and some ligamentum flavum of the patient were cleaned. In addition, the annulus fibrosus was fully exposed while the nerve roots and dural sac were protected. The protruding intervertebral disc tissue was cleaned, and the intervertebral space was treated. Afterwards, the intervertebral cage and broken bone were implanted and compressed appropriately. Finally, the drainage tube was placed after washing the incision, and the incision was sutured layer by layer.

qRT-PCR detection

Peripheral blood was sampled from each patient and centrifuged at 3000 rpm for 10 min within 30 minutes. The total RNA of the serum was acquired via an EasyPure miRNA kit, and its concentration, purity, and integrity were

Table 2. Clinical efficacy grading

Grade	Assessment criteria
Cured	Lumbar and leg pain and numbness of lower limbs disappeared completely, and life and work of the patient returned to normal.
Markedly effective	Lumbar and leg pain and numbness of lower limbs were notably alleviated, and they did not hinder normal life and walking of the patient.
Effective	Pain and numbness of lower limbs were alleviated, but they still existed in the patient.
Ineffective	There was no significant alleviation or aggravation after operation.

Table 3. Comparison of baseline data

Factors	Control group (n = 50)	Patient group (n = 80)	*P*-value
Gender			0.553
Male	28	49	
Female	22	31	
Age (Y)			0.617
≤70	26	38	
>70	24	42	
Past medical history			
Hypertension	20	33	0.888
Hyperlipidemia	10	18	0.736
Smoking history			0.566
Yes	30	52	
No	20	28	
Course of disease (Month)		16.2±8.2	

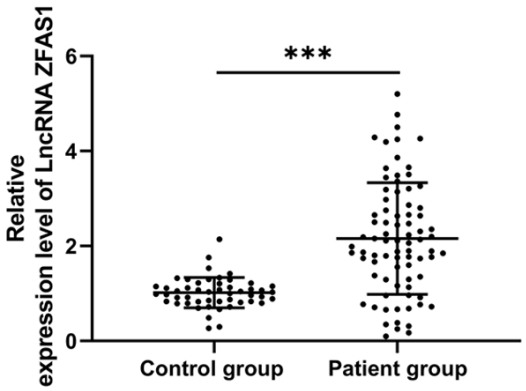

Figure 1. LncRNA ZFAS1 in control group (n = 50) and patient group (n = 80). ***P<0.001.

determined via an ultraviolet spectrophotometer and agarose gel electrophoresis. Subsequently, reverse transcription was conducted on the total RNA via a TransScript® Two-Step RT-PCR SuperMix kit to collect cDNA for PCR amplification under the system configured according to the kit instructions. Amplification system: 1 μL of cDNA, 0.5 μL each of the upstream and downstream primers, 12.5 μL of TransTaq® HIFI PCR SuperMix II, and finally nuclease-free water to a final volume of 25 μL. Amplification conditions: Pre-denaturation (94°C, 3 min), followed by 40 cycles of denaturation (94°C, 30 s), annealing (60°C, 30 s), extension (72°C, 30 s). ZFAS1 was quantified via $2^{-\Delta\Delta CT}$ (internal reference: GAPDH) [15]. Both EasyPure miRNA kit and TransScript® Two-Step RT-PCR SuperMix kit were purchased from TransGen Biotech, Beijing, China. Primer sequences are shown in **Table 1**.

Outcome measures

Primary outcome measures: Serum lncRNA ZFAS1 in the two groups was quantified, and its diagnostic value for LDD was analyzed. In addition, the levels of lncRNA ZFAS1 before therapy and one month after therapy were compared and the associations of lncRNA ZFAS1 with inflammatory factors were analyzed.

Secondary outcome measures: The clinical efficacy grade (**Table 2**) of patients after therapy was evaluated, and the patients were divided into groups according to their clinical efficacy as follows: Cured patients and those with markedly effective treatment were assigned to the good efficacy group, while patients with effective and ineffective treatment were assigned to the general efficacy group. Logistic regression was adopted to analyze risk factors for patients' prognosis and corresponding curves about prediction were drawn.

Statistical analyses

The data of this study were statistically analyzed by SPSS24.0 and visualized by

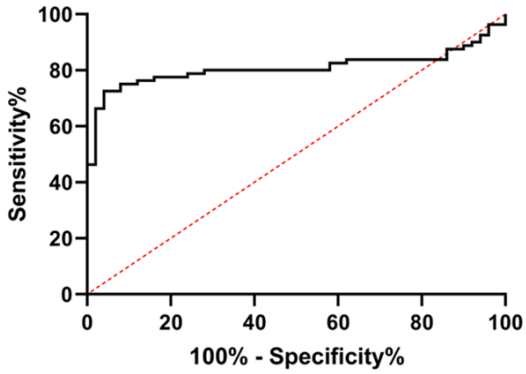

Figure 2. Diagnostic curve of lncRNA ZFAS1 in LDD.

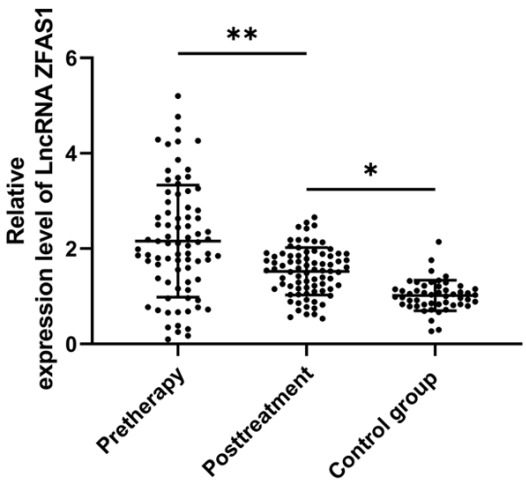

Figure 3. Relative expression of lncRNA ZFAS1 in the patient group (n = 80) before and after treatment, and the comparison with the control group (n = 50) after treatment. **P<0.01.

Graphpad8. Enumeration data were analyzed via the Chi-square test. Measurement data were presented by mean ± SD, and the inter-group comparison was conducted by the independent samples t test. The paired t test was used for the comparison of changes of indicators before and after treatment. Logistic regression was adopted for evaluation of factors affecting patients' prognosis, and receiver operating characteristic (ROC) curve was plotted for value analysis of lncRNA ZFAS1 in predicting LDD. ROC curves of indices with significance in multivariate logistic regression were drawn, and the value of these indices in predicting the clinical efficacy of patients was analyzed. In addition, Pearson's test was adopted for correlation analysis between LncRNA ZFAS1 and inflammatory factors. *P*<0.05 suggested a significant difference.

Results

Comparison of baseline data

The comparison of the clinical baseline data revealed no significant differences between the two groups in gender, age, past medical history and smoking history, indicating that the two groups were comparable (**Table 3**, P>0.05).

LncRNA ZFAS1 presented high expression in patients with LDD

For understanding lncRNA ZFAS1 in patients with LDD, qRT-PCR was carried out to detect serum lncRNA ZFAS1 in the two groups. According to the results, the patient group presented a remarkably higher level of lncRNA ZFAS1 than the control group (**Figure 1**, P<0.001).

LncRNA ZFAS1 was a potential marker for LDD diagnosis

To further determine the diagnostic value of lncRNA ZFAS1 in patients with LDD, we drew corresponding ROC curves. According to the analysis, the area under the curve (AUC) of lncRNA ZFAS1 in diagnosing LDD was 0.807, and its specificity, sensitivity, Youden index, and cutoff value were 96.00%, 72.50%, 68.50%, and 1.545, respectively (**Figure 2**).

Serum lncRNA ZFAS1 in patients decreased after therapy

In this study, we also compared serum lncRNA ZFAS1 in patients before and after therapy. According to the results, patients showed a notable decrease in serum lncRNA ZFAS1 after therapy (P<0.01). Further comparison revealed that the expression level of lncRNA ZFAS1 in patients after therapy was still significantly different from that in the control group (**Figure 3**, P<0.05).

LncRNA ZFAS1 was associated with serum inflammatory factors in patients with LDD

To deeply understand the association of lncRNA ZFAS1 with LDD, we quantified lncRNA ZFAS1 in patients before therapy and analyzed its association with serum TNF-α, IL-6 and IL-1β

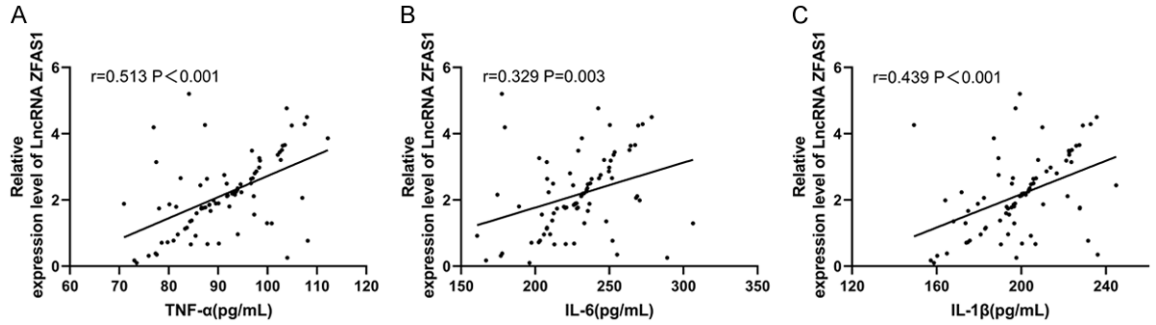

Figure 4. Association of lncRNA ZFAS1 level with TNF-α, IL-6 and IL-1β levels before therapy. A. Correlation analysis of serum lncRNA ZFAS1 level and TNF-α level before treatment (n = 80). B. Correlation analysis of serum lncRNA ZFAS1 level and IL-6 level before treatment (n = 80). C. Correlation analysis of serum lncRNA ZFAS1 level and IL-1β level before treatment (n = 80).

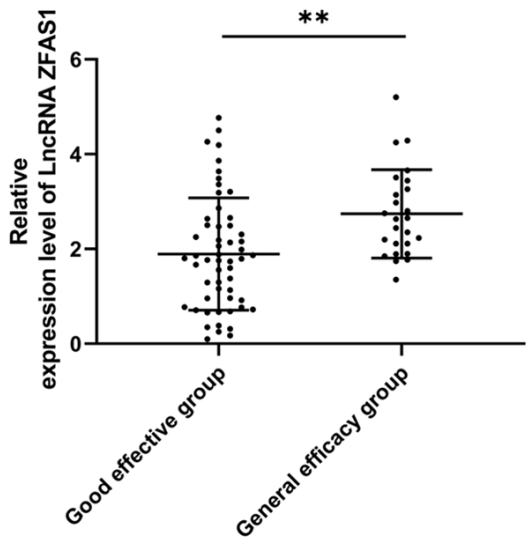

Figure 5. LncRNA ZFAS1 in patients with different curative effects before therapy, good efficacy group (n = 55), general efficacy group (n = 25). **P<0.01.

before therapy. The analysis revealed that lncRNA ZFAS1 was positively associated with TNF-α, IL-6 and IL-1β in patients' serum (**Figure 4A-C**, all P<0.05).

LncRNA ZFAS1 level in patients with favorable efficacy was low before therapy

According to the clinical efficacy after therapy, the patients were divided into two groups, the good efficacy group (n = 55) and the general efficacy group (n = 25), to compare their lncRNA ZFAS1 levels before therapy. It was found that the good efficacy group showed notably lower pre-treatment serum lncRNA ZFAS1 than the general efficacy group (**Figure 5**, P<0.01).

LncRNA ZFAS1 was an independent risk factor for LDD prognosis

Finally, the clinical data of the patients and lncRNA ZFAS1 expression were assigned for logistic regression to explore risk factors affecting the prognosis (**Table 4**). The univariate analysis showed that age, lncRNA ZFAS1 expression before therapy, and disease duration were risk factors for the prognosis. Then, the indices with significant differences in univariate analysis were forwarded to multivariate LR regression, and the results showed that age and lncRNA ZFAS1 expression before therapy were independent risk factors affecting the prognosis of patients (**Table 5**, both P<0.05). Through ROC curves, we confirmed that age and lncRNA ZFAS1 expression before therapy were potential prognostic markers of LDD (**Figure 6A**, **6B**).

Discussion

As a crucial support for the trunk of the body, lumbar vertebra plays an irreplaceable part in the normal activities of the human body [16]. Clinically, LDD is mainly treated by surgery [17]. One study has pointed out that by observing effective predictive indices, early intervention can be achieved to improve the prognosis of patients [18]. However, there is a lack of ideal prognostic indicators for LDD. Thus, finding potential predictors of LDD is of great importance to improve patients' outcomes.

It is shown that LDD is caused by factors such as nutritional disorders, release of inflammatory transmitters, gene mutations, effects of cytokines, and changes in cell matrix [19-21].

Table 4. Assignment

Factors	Assignment
Gender	Male = 1; Female = 2
Age (Y)	≤70 = 1; >70 = 2
Hypertension	Yes = 1; No = 2
Hyperlipidemia	Yes = 1; No = 2
Disease duration (Month)	≤16 = 1; >16 = 2
LncRNA ZFAS1 before therapy	≤1.545 = 1; >1.545 = 2
Efficacy	The good efficacy group = 1; the general efficacy group = 2

Table 5. Logistic regression analysis

Factors	Univariate analysis			Multivariate analysis		
	OR value	*P*-value	95 CI%	OR value	*P*-value	95 CI%
Gender	0.502	0.187	0.181-1.397			
Age	0.222	0.004	0.079-0.623	0.305	0.042	0.097-0.956
Hypertension	0.527	0.191	0.202-1.375			
Hyperlipidemia	0.884	0.829	0.289-2.705			
Disease duration	3.800	0.014	1.316-10.971	2.985	0.071	0.913-9.765
LncRNA ZFAS1 before therapy	5.444	0.005	1.652-17.946	5.586	0.008	1.578-19.778

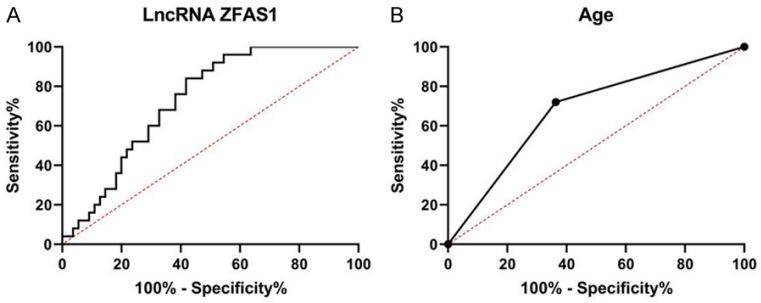

Figure 6. Value of lncRNA ZFAS1 (A) and age (B) in predicting patients' efficacy before therapy.

Some studies have revealed the involvement of lncRNA in LDD development. For instance, lncRNA HOTAIR alleviates degenerative changes of intervertebral disc via the Wnt/β-catenin axis [22]. LncRNA ZFAS1 is a newly discovered lncRNA. Earlier studies have revealed its involvement in various tumors and its influence on disease development via modulating associated inflammatory pathways [23, 24]. However, its clinical and prognostic value in LDD is still under exploration. In our study, lncRNA ZFAS1 showed high expression in patients with LDD and demonstrated a high diagnostic value. With the aim to more deeply understand the significance of lncRNA ZFAS1 in LDD, we carried out a Pearson's correlation analysis and revealed a positive association between LncRNA ZFAS1 and TNF-α, IL-6 and IL-1β in patients' serum, suggesting the involvement of lncRNA ZFAS1 in the development of LDD. The results were consistent with those obtained by Deng et al. [25], and they are mutually verified. Moreover, we further analyzed the prognostic value of lncRNA ZFAS1 in LDD.

In a study by Zheng et al. [26], lncRNA ZFAS1 in rheumatoid arthritis synovial cells was increased significantly by the inducement of TNF-α in the cells, but its knockdown alleviated the inflammatory reaction of rheumatoid arthritis and reduced the release of inflammatory factors. In our study, serum lncRNA ZFAS1 in patients decreased significantly after therapy. We inferred that the long-term compression of intervertebral disc was alleviated after surgical therapy which led to the alleviation of inflammatory reaction in patients and the decreases in inflammatory factor releasing and lncRNA ZFAS1 expression in patients. For the purpose of understanding the association of lncRNA ZFAS1 with patients' prognosis, we grouped the patients according to their clinical efficacy after therapy. The result showed that patients with high lncRNA ZFAS1 level before therapy had poor prognosis. Through logistic regression

analysis, we found that lncRNA ZFAS1 was an independent factor for the prognosis of patients with LDD. The analysis demonstrated the high value of lncRNA ZFAS1 in predicting the efficacy of LDD.

However, this study still has some limitations. First of all, we didn't evaluate the difference of serum lncRNA ZFAS1 between patients with LDD and those with lumbar disc herniation, so whether lncRNA ZFAS1 can be adopted as a bioindicator to identify both LDD and lumbar disc herniation needs further experiments. Second, early studies have revealed the participation of lncRNA in the occurrence of diseases via multiple downstream microRNAs, but the mechanism of lncRNA ZFAS1 in LDD needs to be further investigated. Third, this study did not collect the changes in serum inflammatory factors after treatment. It is still unclear whether there is a correlation between serum inflammatory factor levels and lncRNA ZFAS1 after treatment. Finally, in early studies, both exosomes and peripheral blood mononuclear cells were found to have lncRNAs, but whether detecting LDD in these samples is highly accurate still needs further confirmation. Therefore, we hope to carry out more experiments in the follow-up research to improve our research results.

To sum up, lncRNA ZFAS1 is highly expressed in patients with LDD, and it can be used as a potential prognostic indicator for LDD.

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Disclosure of conflict of interest

None.

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