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Long non-coding RNA zinc finger antisense 1 expression associates with increased disease risk, elevated disease severity and higher inflammatory cytokines levels in patients with lumbar disc degeneration

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Abstract

This study aimed to investigate the correlation of long noncoding RNA zinc finger antisense 1 (lncRNA ZFAS1) expression with disease risk, disease severity and inflammatory cytokines levels in lumbar disc degeneration (LDD) patients.

83 LDD patients underwent surgery and 28 traumatized, non-LDD patients underwent lumbar disc surgery (controls) were consecutively enrolled in this case-control study. Lumbar disc tissue was obtained during surgery and herniated nucleus pulposus (HNP) was isolated to detect lncRNA ZFAS1 expression and inflammatory cytokines mRNA levels by RT-qPCR, and determine protein levels of inflammatory cytokines by western blot.

HNP lncRNA ZFAS1 expression in LDD patients was up-regulated compared with controls ($P<.001$), and receiver operating characteristic (ROC) curve showed lncRNA ZFAS1 expression disclosed a good predictive value for LDD risk with area under curve (AUC) 0.753 (95% CI 0.646–0.859). And after adjustment by age, gender and body mass index (BMI), lncRNA ZFAS1 ($P=.017$) remained to be an independent predictive factor for higher LDD risk. In addition, lncRNA ZFAS1 expression was positively associated with Modified Pfirrmann Grade ($P=.015$). As to inflammatory cytokines, lncRNA ZFAS1 expression was observed to be positively correlated with TNF-α ($P=.002$), IL-1β ($P=.007$) and IL-6 ($P=.015$) mRNAs expressions while reversely associated with IL-10 mRNA level ($P=.014$); and lncRNA ZFAS1 expression was also positively correlated with protein levels of TNF-α ($P=.038$) and IL-6 ($P=.027$) while reversely associated with IL-10 protein expression ($P=.039$).

lncRNA ZFAS1 expression associates with increased risk, elevated disease severity and higher inflammatory cytokines levels in LDD patients.

Abbreviations: AMI = acute myocardial infarction, BCA = bicinchoninic acid, BMI = body mass index, CEL = chemiluminescence, CRP = C-reactive protein, ECM = extracellular matrix, FLS = fibroblast-like synoviocytes, HIF1A = hypoxia-inducible factor 1-alpha, HNP = herniated nucleus pulposus, HNPC = human nucleus pulposus cell, HRP = horse radish peroxidase, IDD = intervertebral disc degeneration, LDD = lumbar disc degeneration, lncRNA ZFAS1 = long noncoding RNA zinc finger antisense 1, MMP = matrix metalloproteinase, PVDF = polyvinylidene fluoride, RA = rheumatoid arthritis, ROC = receiver operating characteristic.

Keywords: inflammatory cytokines, lncRNA ZFAS1, lumbar disc degeneration, risk, severity

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RD and TH contributed equally to this work.

The authors declare that they have no conflicts of interest.

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1. Introduction

Lumbar disc degeneration (LDD), a musculoskeletal disease which accounts for 44.2% of all lumbar disc diseases, is characterized by low back pain and acute lower extremity radicular pain, which is a major source of disability that not only affects the life quality of patients but also brings a huge medical and economic burden to society.[1] The etiology of LDD is complex that accumulating evidences reveal age, gender, environmental, behavioral influences, inflammation and heredity are correlated with LDD risk, among which chronic inflammation is considered as one of important triggers or consequences of LDD[2,3]: One research suggests that variations in inflammation and stromal degradation genes correlate with the severity of LDD, pain score, and disability risk[4]; and another study illustrates that interleukin (IL) 20 level is overexpressed in degenerative disc disease patients compared with spinal fractures patients, and it may promote the degeneration of lumbar disc by affecting the synthesis of proteoglycan.[5]

Long non-coding RNAs (lncRNAs), as a part of non-coding RNAs, contain over 200 nucleotides, even more than 100,000, are involved in lots of crucial biological functions including shaping chromosome conformation, regulating enzymatic activity and so on.[6] Growing evidences have revealed that lncRNAs are concerned with numerous diseases, including cancers, inflammatory diseases and degenerative disease.[7–9] lncRNA zinc finger antisense 1 (ZFAS1), which is frequently investigated in various cancers as an oncogene lncRNA, is recently reported to promote migration and invasion of rheumatoid arthritis (RA) fibroblast-like synoviocytes (FLS) and increase C-reactive protein (CRP) level by targeting miR-150 in acute myocardial infarction (AMI) rat, these suggest that lncRNA ZFAS1 might act as a pro-inflammatory gene which might be involved in the development and progression of LDD.[10,11] Therefore, this study aimed to investigate the correlation of lncRNA ZFAS1 expression with disease risk, disease severity and inflammatory cytokines levels in LDD patients.

2. Materials and methods

2.1. Participants

A total of 83 LDD patients underwent surgery from Jan 2014 to Jun 2017, were consecutively enrolled in this case-control study. The inclusion criteria were as follows:

(1) Diagnosed as LDD according to medical history, clinical examination and imaging;
(2) Age above 18 years;
(3) Herniated nucleus pulposus (HNP) was confirmed as degeneration by historical examination.

The exclusion criteria were as follows:

(1) History of spinal trauma, metabolic bone disease, spinal infection, and spinal deformity;
(2) History of spinal surgery;
(3) History of autoimmune disease, systematic inflammatory disease, solid tumor, or hematological malignancies;
(4) Complicated with severe osteoporosis, severe hepatic, or renal dysfunction;
(5) Women in pregnancy or lactation.

Meanwhile, 28 traumatized, non-LDD patients underwent lumbar disc surgery were recruited in the same period as controls. The inclusion criteria were:

(1) Lumbar disc traumatized, and non-LDD patients confirmed by historical examination of HNP;
(2) Age above 18 years.

The exclusion criteria were:

(1) History of autoimmune disease, systematic inflammatory disease, solid tumor or hematological malignancies;
(2) Complicated with severe osteoporosis, severe hepatic or renal dysfunction;
(3) Women in pregnancy or lactation.

The Ethics Committee of the Central Hospital of Wuhan approved this study, and informed consent was obtained from each participant.

2.2. Data collection and clinical assessment

The demographic information concerning age, gender and body mass index (BMI) were collected from all participants after they were enrolled in this present study. In addition, Modified Pfirrmann grade of LDD patient was evaluated by two orthopedic surgeons specialized in spinal diseases according to the Modified Pfirrmann grading system.

2.3. Sample collection

Lumbar disc tissue from each participant was obtained during the surgical operation, and fibrous ring, cartilage and bone snap were immediately removed. Subsequently, HNP was collected and stored in liquid nitrogen for the further detection of RNA and protein expression.

2.4. Total RNA isolation and cDNA synthesis

To assess the expression of lncRNA ZFAS1 and inflammatory cytokines mRNA including tumor necrosis factor alpha (TNF-α), IL-1β, IL-6, IL-8, and IL-10. The total RNA was isolated using TRIzol reagents (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. RNA concentration and purity were assessed using the DN-1000 Nanodrop Spectrophotometer (Thermo Scientific, Massachusetts, USA). RNA integrity was analyzed using the agarose gel electrophoresis method. 1 μg of total RNA from each sample was used for cDNA synthesis using transcription kit (TOYOBO, Osaka, Japan) following the manufacturer's instructions. The cDNA samples were then diluted 1/20 in RNase Free Water and stored at −20°C until further analysis.

2.5. Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The expression of lncRNA ZFAS1 and inflammatory cytokines mRNA were evaluated by RT-qPCR. cDNA products were subjected to qPCR with SYBR FAST Master mix kit (KAPA, Boston, MA). U6 was used as reference gene for lncRNA ZFAS1 and GAPDH was used as reference gene for mRNAs. We performed the amplification of the cDNA (three technical replicates) per reference gene and sample on the same qPCR plate in 40 cycles. The PCR amplification was performed as follows: initial heat activation at 95°C for 5 minutes, then per cycle denaturation at 95°C for 5 seconds, annealing and extending at 61°C for 30 seconds. Then the qPCR results were calculated with the $2^{-\Delta\Delta Ct}$ method. The sequences of primers were presented in Table 1.

Table 1

Primers sequences.

Gene name	Sequence (5'→3')
LncRNA ZFAS1	F: AAGCCACGTGCAGACATCTA R: CTACTTCCAACACCCGCATT
TNF-α	F: TTCCTCAGCCTCTTCTCCTTCCT R: ATCTCTCAGCTCCACGCCATTG
IL-1β	F: ATCTGTACCTGTCCTGCGTGTTG R: TTCTGCTTGAGAGGTGCTGATGT
IL-6	F: GGTACATCCTCGACGGCATCTC R: GCTCTGGCTTGTTCCTCACTACT
IL-8	F: CTCTTGGCAGCCTTCCTGATTTCT R: CGCAGTGTGGTCCACTCTCAAT
IL-10	F: TGTTGCCTGGTCCTCCTGACT R: GCCTTGATGTCTGGGTCTTGGTT
GAPDH	F: TGACCACAGTCCATGCCATCAC R: GCCTGCTTCACCACCTTCTTGA
U6	F: CTCGCTTCGGCAGCACATATACTA R: ACGAATTTGCGTGTCATCCTTGC

2.6. Western blot assay

Total proteins were extracted from HNP by RIPA buffer (Thermo Fisher Scientific, MA), the total protein concentration in each sample was measured using the bicinchoninic acid (BCA) kit (Price Biotechnology, Rockford, IL) and compared with the standard curve. Next, 20 ug total proteins were electrophoretically transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, MA). After blocking with 5% skim milk for 24 hours, membranes were incubated with the corresponding primary antibody overnight at 4°C. Then, membranes were incubated with the appropriate horse radish peroxidase (HRP)-conjugated secondary antibody for 1 hour at room temperature. The bands were visualized using an enhanced chemiluminescence (CEL) kit (Millipore, MA) followed by exposure to X-ray film. All experiments were triplicated. The Image J Software (Java, USA) was used to determine the density of results from western blot. The ratio of the density of the target protein and GAPDH was calculated for evaluating the protein expressions. The antibody information was presented in Table 2.

2.7. Statistical analysis

Statistical analysis was performed by SPSS Software 22.0 (IBM, Chicago, IL) and GraphPad Prism 6 (IBM, New York, NY). Data was presented as mean ± standard deviation, median (25th–75th) or count (%). Comparison of basic characteristics between LDD patients and controls was determined by student test or Chi-square test. Difference of the lncRNA ZFAS1 expression between LDD patients and controls was determined by Wilcoxon rank-sum test. Receiver operating characteristic (ROC) curve was conducted to assess the predictive value of lncRNA ZFAS1 expression for LDD risk. Univariate and multivariate logistic regression analyses were performed to adjust the influence of age, gender and BMI on lncRNA ZFAS1 expression for predicting LDD risk. The Spearman correlation analysis was used to assess the correlation of lncRNA ZFAS1 expression with Modified Pfirrmann Score, 5 inflammatory cytokines mRNAs and proteins levels. As for the sample size calculation, in our preliminary study, we observed that the LDD patients and control patients treated in our hospital were at 3:1 ratio, meanwhile lncRNA ZFAS1 was about 1.9 ± 1.1 in LDD patients while 1.0 ± 0.6 in control patients; Using a power of 80% and a two-sided 5% level of significance (α) to detect a difference of lncRNA ZFAS1 between the two groups, a smallest sample size of 39 LDD patients and 13 controls were required, while in order to further improve the statistical power, we enrolled 83 LDD patients and 28 controls in this present study. *P* value <.05 was considered significant.

Table 2

Antibody information.

Antibody name	Company	Country	Dilution ratio
Primary antibody			
Rabbit polyclonal Anti-TNF-α antibody	Abcam	USA	1:2000
Rabbit polyclonal IL-1β antibody	Abcam	USA	1:2000
Rabbit polyclonal IL-6 antibody	Abcam	USA	1:2000
Rabbit polyclonal Anti-IL-8 antibody	Abcam	USA	1:2000
Rabbit polyclonal IL-10 antibody	Abcam	USA	1:2000
Rabbit polyclonal GAPDH antibody	Abcam	USA	1:2000
Second antibody			
Goat Anti-Rabbit IgG H&L	Abcam	USA	1:2000

3. Results

3.1. Basic characteristic of LDD patients and controls

As listed in Table 3, the mean age in 28 controls and 83 LDD patients was 33.3 ± 9.5 years and 48.1 ± 5.0 years (*P* < .001). Among them, there were 15 males (53.6%) and 13 females

Table 3

Basic characteristics of LDD patients and controls.

Parameters	Controls (N = 28)	LDD patients (N = 83)	*P* Value
Age (yr)	33.3 ± 9.5	48.1 ± 5.0	**<.001**
Gender (n/%)			.872
Male	15 (53.6)	43 (51.8)	
Female	13 (46.4)	40 (48.2)	
BMI (kg/m^2)	23.31 ± 2.86	24.37 ± 3.45	.146
Modified Pfirrmann Grade			–
Grade 3	–	27 (32.5)	
Grade 4	–	21 (25.3)	
Grade 5	–	24 (28.9)	
Grade 6	–	10 (12.1)	
Grade 7	–	1 (1.2)	
TNFα			–
mRNA relative expression	–	2.095 (1.301–2.921)	
protein relative level	–	0.609 (0.420–0.461)	
IL-1β			–
mRNA relative expression	–	1.538 (0.948–1.988)	
protein relative level	–	0.563 (0.367–0.757)	
IL-6			–
mRNA relative expression	–	1.969 (1.430–2.908)	
protein relative level	–	0.571 (0.457–0.765)	
IL-8			–
mRNA relative expression	–	1.768 (1.216–1.799)	
protein relative level	–	0.437 (0.331–0.565)	
IL-10			–
mRNA relative expression	–	2.458 (1.541–3.298)	
protein relative level	–	0.537 (0.390–0.672)	

Data was presented as mean value ± standard deviation, median (quartile 25th–75th) or count (%). Comparison was determined by *t* test or Chi-square test. *P* Value <.05 was considered significant. BMI = body mass index, IL = interleukin, LDD = lumbar disc degeneration, TNF = tumor necrosis factor.

(46.4%) in controls, 43 males (51.8%) and 40 females (48.2%) in LDD patients ($P=.872$). Besides, the BMI mean value in controls and LDD patients were 23.31 ± 2.86 kg/m^2 and 24.37 ± 3.45 kg/m^2 ($P=.146$). The number of LDD patients in Modified Pfirrmann Grade 3, Grade 4, Grade 5, Grade 6, and Grade 7 were 27 (32.5%), 21 (25.3%), 24 (28.9%), 10 (12.1%) and 1 (1.2%), respectively. The detailed information of inflammatory cytokines expression in HNP of LDD patients were presented in Table 3.

3.2. lncRNA ZFAS1 expression in LDD patients and controls

Wilcoxon rank-sum test was used to analyze difference of the lncRNA ZFAS1 expression between LDD patients and controls, which showed that the expression of lncRNA ZFAS1 in LDD patients (1.768 (0.976–3.627)) was up-regulated compared with controls (0.759 (0.475–1.830)) ($P<.001$, Fig. 1).

3.3. Predictive value of lncRNA ZFAS1 expression for LDD risk

In order to further investigate the value of lncRNA ZFAS1 expression in predicting LDD risk, ROC curve was performed. As shown in Figure 2, lncRNA ZFAS1 disclosed a good predictive value for LDD risk with area under curve (AUC) 0.753 (95% CI 0.646–0.859), and sensitivity was 88.0% as well as specificity was 53.6.0% respectively at the best cut-off point (defined as the point at which the value was largest by adding sensitivity to specificity).

3.4. The effect of lncRNA ZFAS1 on LDD risk after adjustment by age, gender and BMI

Univariate and multivariate logistic regression analyses were used to adjust the influence of age, gender and BMI on lncRNA ZFAS1 expression for predicting LDD risk. As shown in Table 4, univariate logistic regression showed that lncRNA ZFAS1 ($P=.001$) and age ($P<.001$) were correlated with LDD risk. All factors with P value <.1 were included in the multivariate logistic regression analysis, which displayed that lncRNA ZFAS1 ($P=.017$) remained to be the independent predictive factors for LDD risk after adjusted by age, gender and BMI. In addition, age ($P<.001$) was also independently associated with LDD risk.

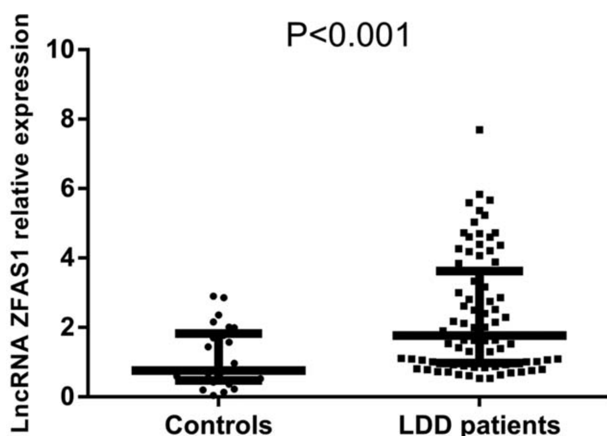

Figure 1. The lncRNA ZFAS1 expression in LDD patients and controls. The expression of lncRNA ZFAS1 was increased in LDD patients compared with controls. Comparison between two groups was determined by Wilcoxon rank sum test. $P<.05$ was considered significant.

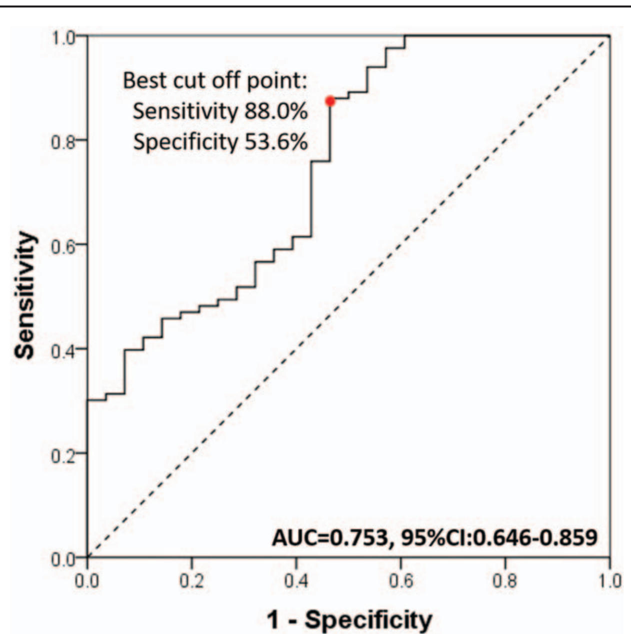

Figure 2. The ROC curve of lncRNA ZFAS1 expression for predicting LDD risk. ROC curve showed lncRNA ZFAS1 had a good AUC for predicting LDD risk.

3.5. Correlation of lncRNA ZFAS1 expression with modified Pfirrmann grade

The Spearman correlation analysis was performed to assess the association of lncRNA ZFAS1 expression with Modified Pfirrmann Score and the result found that lncRNA ZFAS1 expression was positively associated with Modified Pfirrmann Grade (r = 0.267, $P=.015$, Fig. 3).

3.6. Correlation of lncRNA ZFAS1 expression with inflammatory cytokines mRNA level

We discovered that lncRNA ZFAS1 expression was positively correlated with mRNA expressions of TNF-α (r = 0.338, $P=.002$, Fig. 4A), IL-1β (r = 0.295, $P=.007$, Fig. 4B) and IL-6 (r = 0.267, $P=.015$, Fig. 4C), while reversely associated with IL-10 mRNA expression (r = −0.270, $P=.014$, Fig. 4E). However, no correlation between lncRNA ZFAS1 expression and IL-8 mRNA expression (r = 0.085, $P=.190$, Fig. 4D) was observed.

3.7. Correlation of lncRNA ZFAS1 expression with inflammatory cytokines protein levels

We performed a density quantification of the western blot results (Fig. 5F) to analyze the association between lncRNA ZFAS1 expression and inflammatory cytokines protein levels, and discovered that the results were mostly consistent with mRNA results: lncRNA ZFAS1 expression was positively correlated with protein expressions of TNF-α (r = 0.228, $P=.038$, Fig. 5A) and IL-6 (r = 0.243, $P=.027$, Fig. 5C) while reversely associated with IL-10 protein expression (r = −0.227, $P=.039$, Fig. 4E); But no

Table 4

Logistic regression analysis of factors for LDD risk.

	Univariate logistic regression (N=111)				Multivariate logistic regression (N=111)			
	P value	OR	95% CI		*P* value	OR	95% CI	
			Lower	Higher			Lower	Higher
LncRNA ZFAS1	**.001**	2.338	1.393	3.923	**.017**	2.651	1.188	5.919
Age	**<.001**	1.352	1.202	1.520	**<.001**	1.352	1.192	1.534
Gender (male)	.872	0.932	0.395	2.198	.731	0.775	0.181	3.316
BMI	.148	1.101	0.966	1.255	.518	1.084	0.849	1.384

Data was presented as *P* value, OR (odds ratio) and 95% CI (confidence interval). Significance was determined by univariate and multivariate logistic regression analysis. *P* value <.05 was considered significant. BMI=body mass index, LDD=lumbar disc degeneration.

correlation of lncRNA ZFAS1 expression with IL-1β (r=0.204, *P*=.064, Fig. 5B) and IL-8 (r=0.164, *P*=.139, Fig. 5D) protein expressions was discovered.

4. Discussion

In our study we found:

(1) HNP lncRNA ZFAS1 expression was dramatically increased in LDD patients compared with controls, and it had a good predictive value for LDD risk, besides, after adjusting the influence of age, gender and BMI, lncRNA ZFAS1 was an independently predictive factor for LDD risk;
(2) lncRNA ZFAS1 expression was positively associated with Modified pfirrmann grade;
(3) lncRNA ZFAS1 expression was positively correlated with TNF-α, IL-1β as well as IL-6 expressions, but was negatively associated with IL-10 expressions.

lncRNAs, playing important roles in multiple processes of human biology, are key regulators in epigenetics as recruiters, tethers and scaffolds that involve mRNA processing, stability and post-transcriptional regulation translation.[12,13] The dysregulation of lncRNAs is reported to be associated with various diseases, including degenerative diseases. For example, Zhang et al illuminates that upregulation of lncRNA HOX transcript antisense RNA (HOTAIR) promotes IL-1β-induced matrix metalloproteinase (MMP) over expression and chondrocytes apoptosis in temporomandibular joint osteoarthritis.[14] And another study shows that lncRNA RP11–296A18.3 promotes hypoxia-inducible factor 1-alpha (HIF1A) expression through sponging miR-138, thus promotes human nucleus pulposus cell (HNPC) proliferation and extracellular matrix (ECM) synthesis in intervertebral disc degeneration (IDD).[15] These suggest that lncRNAs might play an important role in occurrence or development of degenerative diseases.

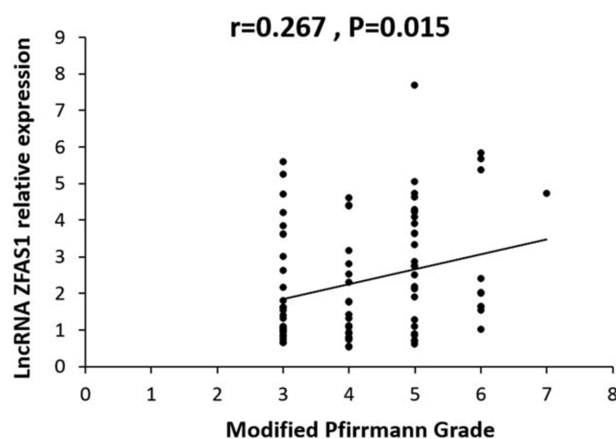

Figure 3. The association of lncRNA ZFAS1 expression with the Modified pfirrmann grade. The level of lncRNA ZFAS1 was positively correlated with Modified pfirrmann grade. The correlation of lncRNA ZFAS1 expression with Modified pfirrmann grade was determined by Spearman correlation analysis. *P*<.05 was considered significant.

The etiology of LDD is complex, previous studies suggest that heavy physical load, which often associates with occupation, is the main suspected risk factor.[16] In addition to the heavy physical load, inflammation is illustrated to be correlated with LDD risk as well. In recent years, some studies reveal that inflammatory cytokines were abnormally expressed in LDD patients. A study shows that the mRNA and protein levels of IL-1α in LDD patients are markedly increased compared with controls, and the IL-1α level is positively correlated with the disease severity.[17] In addition, the study of Omair et al illuminates that variation in IL-18 receptor genes elevates the risk of severe LDD and associates with low back pain.[18] These studies suggest that the abnormal expressions of inflammatory cytokines might be involved in the occurrence or development of LDD.[17,18]

lncRNA ZFAS1, located on human chromosome 20q13.13, whose functions are mainly investigated in the cancers pathogenesis recently. Several studies illuminate that lncRNA ZFAS1 promotes cells proliferation, invasion and metastasis in colorectal cancer, osteosarcoma and gastric cancer through regulating multiple target miRNA, genes or pathways including kruppel-like factor 2 (KLF2), zinc finger e-box-binding homeobox 2 (ZEB2), miR-484.[19–21] However, there are only a few reports exploring the role of lncRNA ZFAS1 in inflammation-related diseases or degenerative diseases. One study exhibits that knockdown of lncRNA-ZFAS1 protects cardiomyocytes against AMI via promoting anti-apoptosis by regulating miR-150/CRP, thus effectively improves myocardial infarction in rat AMI model.[11] Another study shows that compared with healthy donors, lncRNA ZFAS1 expression was increased in synovial tissue and FLS from RA patients, and further experiments reveals that lncRNA ZFAS1 promotes cells migration and invasion of RA-FLS by suppression of miR-27a, implying lncRNA ZFAS1 might enhance inflammatory responses in RA patients.[10] These indicate that lncRNA ZFAS1 dysregulations might be closely correlated with inflammation which involve in the development and progression of LDD. However, no research has been done to investigate the role of lncRNA ZFAS1 in LDD pathogenesis until now. In this present study, we found lncRNA ZFAS1 expression was elevated in LDD patients compared with controls, and it had a good diagnostic value for LDD with AUC 0.753 (95% CI

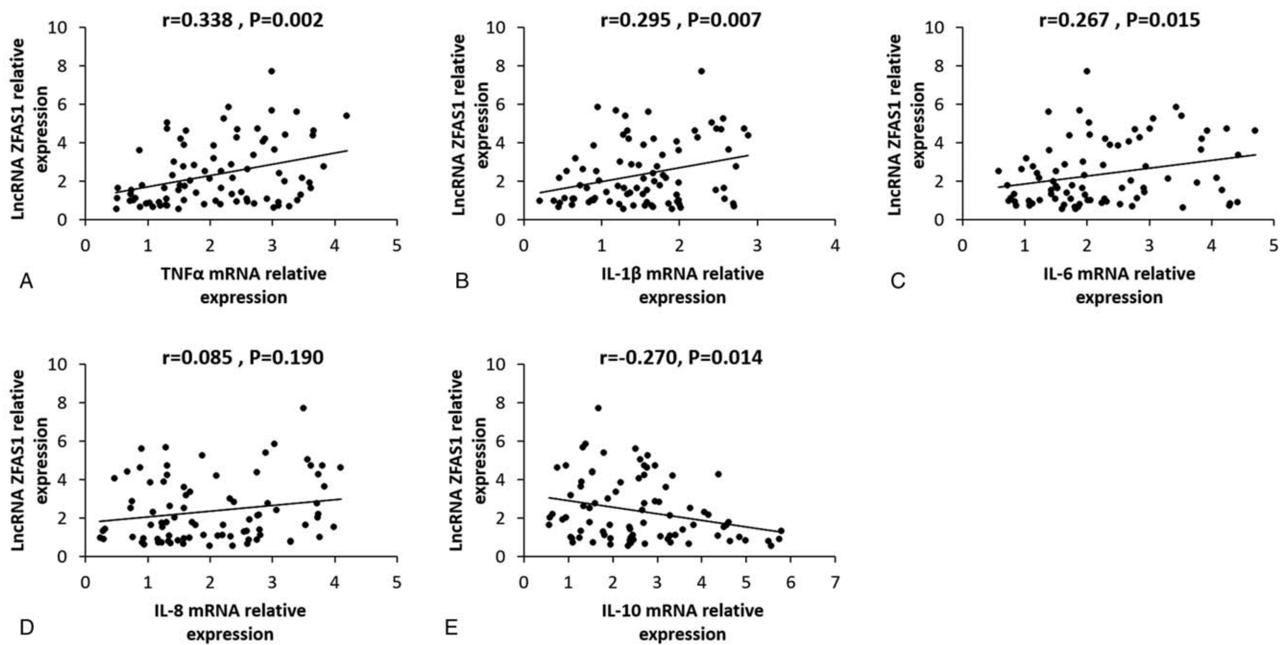

Figure 4. Correlation of lncRNA ZFAS1 expression with mRNA levels of inflammatory cytokines. lncRNA ZFAS1 expression was positively correlated with mRNA levels of TNF-α (A), IL-1β (B) and IL-6 (C), no correlation of lncRNA ZFAS1 level with IL-8 mRNA expression (D) was discovered. And a negative correlation of lncRNA ZFAS1expression with IL-10 mRNA level was observed (E). The correlation of lncRNA ZFAS1 expression with mRNA levels of inflammatory cytokines was determined by Spearman correlation analysis. $P < .05$ was considered significant.

0.646–0.859). In addition, after adjustment of age, gender and BMI, lncRNA ZFAS1 expression independently predicted higher LDD risk, the lncRNA ZFAS1 and was the independently predictive factor for LDD risk, and age is also independently associated with LDD risk. These results in our study might be due to that lncRNA ZFAS1 could positively regulate the systematic inflammation leading to the LDD development and disease progression.[10] In addition, a probable explanation of age as an

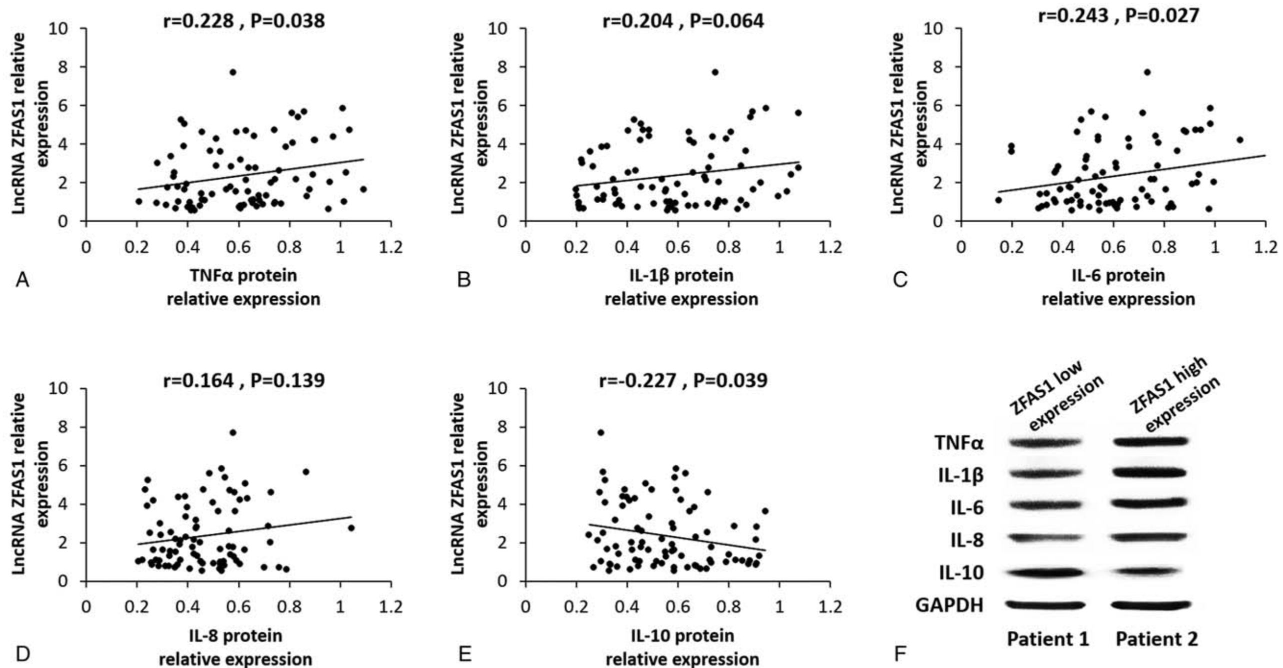

Figure 5. Correlation of lncRNA ZFAS1 expression with the protein level of inflammatory cytokines. lncRNA ZFAS1 level was positively correlated with protein expressions of TNF-α (A) and IL-6 (C), but not associated with IL-1β (B) or IL-8 (D) level. In addition, a negative correlation of lncRNA ZFAS1 expression with IL-10 protein level was observed (E). The correlation of lncRNA ZFAS1 expression with protein levels of inflammatory cytokines was determined by Spearman correlation analysis. $P < .05$ was considered significant.

independent predictor of LDD is that the mechanical stress on the spine of the elderly for many years leads to degeneration in the inherent structure of the disc.[22]

Besides, we also observed that lncRNA ZFAS1 expression was positively associated with Modified pfirrmann grade which was used to evaluate the disease severity of LDD. This might result from that lncRNA ZFAS1 promotes HNP inflammation which accelerates the progression of LDD. In order to further investigate the correlation of lncRNA ZFAS1 expression with inflammation in LDD patients, we detected the mRNA and protein expressions of five inflammatory cytokines in the HNP of LDD patients as well, which disclosed that lncRNA ZFAS1 expression was positively associated with expressions of TNF-α, IL-1β as well as IL-6, while negatively correlated with IL-10 expression. These suggested that lncRNA ZFAS1 was a pro-inflammatory gene in LDD.

Some limitations existed in this study. First, the sample size of this study was relatively small with 83 LDD patients and 28 controls. However, the SNP samples were hard to be obtained in real-world clinical conditions especially from controls. Second, the LDD patients and controls were enrolled only in one center, which could cause selection bias. Thus, a multi-center study with larger sample size is needed in the future. Third, the specific mechanism of lncRNA ZFAS1 in LDD etiology and regulating inflammation has not been studied yet and further in vitro and in vivo experiments are needed.

In conclusion, lncRNA ZFAS1 expression associates with increased risk, elevated disease severity and higher inflammatory cytokines levels in LDD patients.

Author contributions

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Writing – review & editing: Jun Li, Shuang-Bing Feng, Yifan Wang.

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