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Analysis of associations of FBXL19-AS1 with occurrence, development and prognosis of acute pancreatitis

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Abstract. – OBJECTIVE: The aim of the study was to explore the association between F-box and leucine-rich repeat protein 19-antisense ribonucleic acid 1 (FBXL19-AS1) and acute pancreatitis (AP) and its role in prognostic evaluation.

PATIENTS AND METHODS: According to the severity of AP, the patients were classified into mild group, moderate-severe group, and severe group, and the expression of FBXL19-AS1 was compared among the three groups. The associations of FBXL19-AS1 with Atlanta classification, computed tomography severity index (CTSI), acute physiology and chronic health evaluation (APACHE) II score, bedside index for severity in acute pancreatitis (BISAP) and Ranson score were analyzed. The optimal cut-off point of severe hyperlipidemia-induced AP was predicted by the receiver operating characteristic (ROC) curves. Then, the incidence rates of local and systemic complications were compared among AP patients with different levels of FBXL19-AS1. After overexpression of FBXL19-AS1 in AP cells, the quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) results showed that significantly upregulated the mRNA level of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6. The opposite results were obtained after the knockdown of FBXL19-AS1 in cells.

RESULTS: There were no statistical differences in the basic data among the three groups. The FBXL19-AS1 level was increased in severe group than the moderate-severe group and mild group. The area under the curve (AUC) of FBXL19-AS1 in predicting severe AP was 0.9177 ($p<0.001$). According to the Spearman correlation analysis, the FBXL19-AS1 level had significant positive correlations with the predictive scores of AP severity. The incidence rate of shock, liver dysfunction, and pancreatic necrotic tissue infection was significantly higher in FBXL19-AS1 high-expression group than that in FBXL19-AS1 low-expression group. FBXL19-AS1 could promote the upregulation of inflammatory indexes.

CONCLUSIONS: FBXL19-AS1 is highly expressed in the serum of AP patients, and it is positively correlated with the severity of AP. FBXL19-AS1 mediates the inflammatory response and promotes the occurrence and development of pancreatitis, harming the prognosis of patients.

Key Words:
FBXL19-AS1, Occurrence, Prognosis, Acute pancreatitis.

Introduction

Acute pancreatitis (AP) refers to the inflammatory responses, such as pancreatic edema, hemorrhage and necrosis induced by pancreatic self-digestion due to multiple causes, with or without other organ failure, which may lead to death in severe cases[1,2].

Currently, the severity of AP is often evaluated by the acute physiology and chronic health evaluation (APACHE) II score, Ranson score, bedside index for severity in acute pancreatitis (BISAP) and computed tomography severity index (CTSI). However, these scoring criteria contain many items, it is complicated to calculate the APACHE II score, and it takes 48 h to evaluate the Ranson score[3-7]. Moreover, AP tends to develop rapidly, and it is particularly important to distinguish mild AP (MAP) from severe AP (SAP) for the treatment and control of AP. Therefore, it is urgently needed to find a single, effective, and easy-to-operate index to the accurately predicted the severity of AP in the early stage.

Long non-coding ribonucleic acids (lncRNAs) have long been considered as the genomic "junk", so their functions are always ignored. With the rapid development of sequencing technique and genetics in related fields, the biological functions of lncRNAs have been gradually concerned. LncRNAs have a wide range of biological functions in organisms; in particular, crucial functions in the post-transcriptional regulation level, cell cycle, and differentiation regulation[8]. It has been

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observed that B3GALT5-antisense RNA 1 (AS1) exerts key functions in the progression of AP[9]. As one of the most important members of the lncRNA family, FBXL19-AS1 is closely related to development of tumors, and its expression is closely associated with the incidence of colorectal cancer[10]. However, there have been no studies on the correlation between FBXL19-AS1 and AP yet. Therefore, this research aims to uncover the association between FBXL19-AS1 and the severity of AP and its role in prognostic evaluation.

Patients and Methods

Objects of Study

The AP patients admitted in Qinghai University Affiliated Hospital from June 2017 to May 2019 were collected. Inclusion criteria: AP was diagnosed in line with 2 of the following 3 criteria in the 2013 Revision of the Atlanta Classification and Definitions by International Consensus: 1) Abdominal pain conforming to the characteristics of AP; 2) increase in serum lipase or amylase, at least 3 times higher than the upper limit of normal value; 3) typical imaging manifestations of pancreatitis[11]. Based on the Revision of the Atlanta Classification and Definitions by International Consensus, the severity of AP is classified as follows: 1) MAP (no organ failure and local or systemic complications); 2) moderate-severe AP (recovery of organ failure within 48 h, and/or local or systemic complications, and no persistent organ failure); 3) SAP (persistent organ failure >48 h). The exclusion criteria involved: 1) patients with recurrent pancreatitis; 2) those with tumor, blood disease or autoimmune disease; 3) those undergoing chemotherapy or hormone therapy. This study was approved by the Ethics Committee of Qinghai University Affiliated Hospital. All patients agreed and signed the informed consent.

Serum Collection

Peripheral venous blood (2 mL) was drawn from the objects and stored at 4°C for 30 min. Then, the samples were centrifuged at 3,000 rpm for 15 min at 15°C. Then, the upper-layer serum was collected and placed in a refrigerator at -80°C for later use.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The total RNA was extracted according to the protocol of RNA extraction kit (TIANGEN Biotechnology Co., Ltd., Beijing, China). With the RNA as the substrate, it was added with specific RT primers (Applied Biosystems, Foster City, CA, USA) and reversely transcribed according to the instructions of RT kit (Applied Biosystems, Foster City, CA, USA), followed by amplification (95°C for 5 min, 95°C for 10 s, 60°C for 30 s, 72°C for 30 s, with a total of 35 cycles). Primer sequences were designed as shown: FBXL19-AS1 F: 5'-GGT ACA ACT ACG GAT ATG A-3', R: 5'-TAC GTC TCG ACC ATTACGCA-3'. Interleukin-1β (IL-1β), F: 5'-CCCTGAACTCAACTGTGAAATAGCA-3', R: 5'-CCCAAGT-CAAGGGCTTGGAA-3'. IL-6 F: 5'-ATTGTAT-GAACAGCGATGATGCAC-3', R: 5'-CCAGGTAGAAACGGAACTC-CAGA-3'. Tumor necrosis factor-α (TNF-α): F: 5'-TTC-CAATGGGCTTTCGGAAC-3', R: 5'-AGA-CATCTTCAGCAGCCTTGTGAG-3'. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) F: 5'-CGGATTTGGTCGTATTGGG-3', R: 5'-GATTTTGGAGGGATCTCGC-3'.

Cell Culture

The rat pancreatic acinar AR42J cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA), and cultured in minimum essential medium (MEM; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) in an incubator with 5% CO_2 at 37°C.

Establishment of Necrotizing Pancreatitis Cell Model

AR42J cells were digested with trypsin, washed with phosphate buffered saline (PBS), and counted. Then, the suspended cells were seeded into 24-well plates (3×10^4 cells/well) and routinely cultured in the incubator. After 24 h, 200 μM taurolithocholic acid 3-sulfate disodium salt (TLC-S) was added, and the supernatant of culture medium was collected to detect amylase content within 1 h.

Cell Transfection

The cells were firstly digested with trypsin (Gibco, Rockville, MD, USA), and the cell was adjusted to 1×10^5 cells/mL with MEM. Then, 2 mL of cell suspension were inoculated into each 6-well plate and cultured in the incubator with 5% CO_2 at 37°C for 24 h, followed by transfection according to the instructions of Lipofectamine™ 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA). At 6-8 h after transfection, the medium was replaced, with untreated cells as blank

Table I. Comparison of general data among the three groups of AP patients.

Variable	MAP (n=40)	MSAP (n=40)	SAP (n=40)	F/χ^2	p
Age	63.18±7.75	61.23±6.85	62.91±8.08	1.2	0.3
Sex (male/female)	20/20	20/20	20/20	NA	NA
BMI (kg/m^2)	27.13 ± 3.21	28.73 ± 3.01	28.61±3.17	0.21	0.81
Hyperlipidemia [n (%)]	25 (62.5%)	23 (57.5%)	23 (57.5%)	0.28	0.87
Coronary heart disease [n (%)]	2 (5%)	3 (7.5%)	1 (2.5%)	1.05	0.59
Smoking [n (%)]	16 (40%)	13 (32.5%)	20 (50%)	2.55	0.28
Drinking [n (%)]	22 (55%)	24 (60%)	28 (70%)	1.97	0.37

Note: BMI, body mass index.

controls. After 48 h, the old medium was replaced with the medium containing 2 μg/mL puromycin, and the cells were cultured for other 72 h and inoculated into a new 6-well plate. After 1-2 weeks, the colonies formed were screened into a 96-well plate and cultured again. When the colonies covered the bottom of plate, they were transferred into a 6-well plate, and then transferred into a culture flask 2 d later, so as to construct the stable cell lines. The sequences were designed as follows: si-FBXL19-AS1 sense 5'-CCG GCC TCC CTA AGT GTT GGG ATT ACT CGA GTA ATC CCA ACA CTT AGG GAG GTT TTT TG-3', antisense: 5'-CCG GGC ATT TAA TTT GGC ATA GCA ACT CGA GTT GCT ATG CCA AAT TAA ATG CTT TTT TG-3'.

Enzyme-Linked Immunosorbent Assay (ELISA)

The amylase content in cells was determined using the ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the instructions, and the results were analyzed.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM Corp., Armonk, NY, USA) was enrolled for statistical analysis of all data. Categorical data were expressed as cases, and χ^2 test was computed. Measurement data were expressed as mean ± standard deviation. The differences between the two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). The data correlation was evaluated using the Spearman correlation analysis, and the diagnostic efficiency of index was assessed using the receiver operating characteristic (ROC) curve. *p<0.05 suggested that the difference was statistically significant.

Results

Comparison of Basic Data Among the Three Groups of AP Patients

Among the 120 AP patients, there were 40 cases in mild, moderate-severe, and severe group, respectively. There were no statistical differences in gender, age, BMI (body mass index), history of hyperlipidemia, history of coronary heart disease, and history of smoking and drinking among the three groups (p>0.05) (Table I). It can be seen that the general clinical data were balanced and comparable among the three group s.

Correlation Analysis Between FBXL19-AS1 and Predictive Scores of AP

To explore the correlation between FBXL19-AS1 and predictive scores of AP, Spearman correlation regression analysis was performed. We found that the expression level of FBXL19-AS1 was positively correlated with Atlanta classification, APACHE II score, CTSI, BISAP and Ranson score (r=0.352, 0.532, 0.337, 0.194 and 0.443) (Table II). It can be inferred that FBXL19-AS1 may be used to evaluate the severity of AP.

Comparison of Serum FBXL19-AS1 Level Among the Three Groups of AP Patients

To investigate the role of FBXL19-AS1 on AP, the level of FBXL19-AS1 was detected *via* qRT-

Table II. Correlation analysis between FBXL19-AS1 and predictive scores of AP.

Variable	r	p
New Atlanta Standard	0.352	<0.001
APACHE II	0.532	<0.001
CTSI	0.337	0.006
BISAP	0.194	0.021
Ranson	0.443	0.007

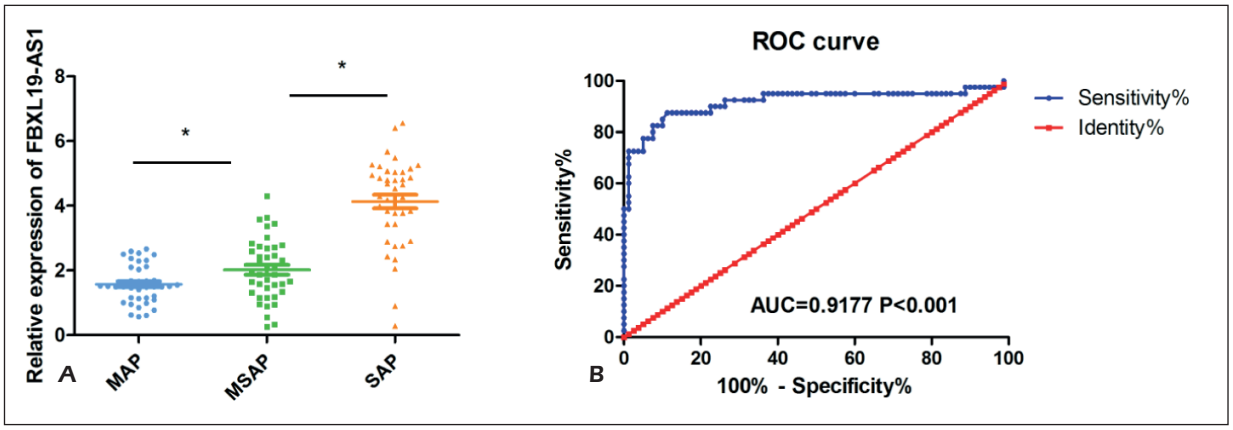

Figure 1. Comparison of serum FBXL19-AS1 level among the three groups of AP patients. **A**, The results of qRT-PCR showed that the level of FBXL19-AS1 had statistically significant differences among the three groups ($p<0.05$). It was higher in severe group than that in mild group and moderate-severe group, and also higher in moderate-severe group than that in mild group ($p<0.05$). **B**, ROC curve analysis of FBXL19-AS1 level in diagnosing severe hyperlipidemia-induced AP.

PCR. The results showed that its level had statistical differences among the three groups, which was increased in severe group compared to the mild and moderate-severe group, and the moderate-severe group was higher than that in the mild group (Figure 1A). The AUC of FBXL19-AS1 in predicting SAP was 0.9177 (95% CI: 0.85-0.98, $p<0.001$) (Figure 1B), indicating that FBXL19-AS1 has the potential to be a biomarker of SAP.

Effects of Different Expressions of FBXL19-AS1 on Local or Systemic Complications

With the median expression of serum FBXL19-AS1 as the cut-off point, the AP patients were classified into high-expression and low-expression group. According to the results, the incidence rate of shock, liver dysfunction, and pancreatic necrotic tissue infection was significantly higher in high level group than that in low level group (Table III), suggesting that highly expressed FBXL19-AS1 may lead to adverse reactions, such as shock, liver dysfunction, and pancreatic necrotic tissue infection in AP patients.

FBXL19-AS1 Promoted Expressions of IL-1β, IL-6 and TNF-α

To further explore the mechanism of FBXL19-AS1 in promoting the occurrence and development of AP, *in vitro* cell experiments were conducted. The AR42J cells were treated with TLC-S to establish the necrotizing pancreatitis cell model, and the amylase content in the supernatant was determined. The results revealed that the amylase content was significantly higher in *in vitro* model group than that in the control group [(1942.5±25.1) *vs.* (287.7±7.3), $p<0.05$], confirming that the necrotizing pancreatitis cell model was successfully induced. Besides, the expression of FBXL19-AS1 in cells was markedly upregulated after overexpression of FBXL19-AS1 (Figure 2A), while it was notably downregulated after the knockdown of FBXL19-AS1 (Figure 2B). It can be indicated that the effect of transfection was good, and subsequent experiments could be done. In addition, the results of qRT-PCR manifested that the mRNA expressions of IL-1β, IL-6 and TNF-α evidently rose after overexpression of FBXL19-AS1

Table III. Effects of different expressions of FBXL19-AS1 on local or systemic complications.

Clinical prognosis	High level (n=60)	Low level (n=60)	χ^2	*p*
Shock [n (%)]	30 (50%)	17 (28.3%)	5.911	0.024
Acute respiratory distress syndrome [n (%)]	19 (31.7%)	14 (23.3%)	1.045	0.414
Acute kidney injury [n (%)]	18 (30%)	14 (23.3%)	0.682	0.536
Liver dysfunction [n (%)]	26 (43.3%)	13 (21.7%)	6.42	0.019
Abdominal hypertension [n (%)]	40 (66.7%)	29 (48.3%)	4.126	0.064
Bleeding [n (%)]	20 (33.3%)	15 (25%)	1.008	0.422
IPN [n (%)]	34 (56.7)	16 (26.7%)	11.109	0.002

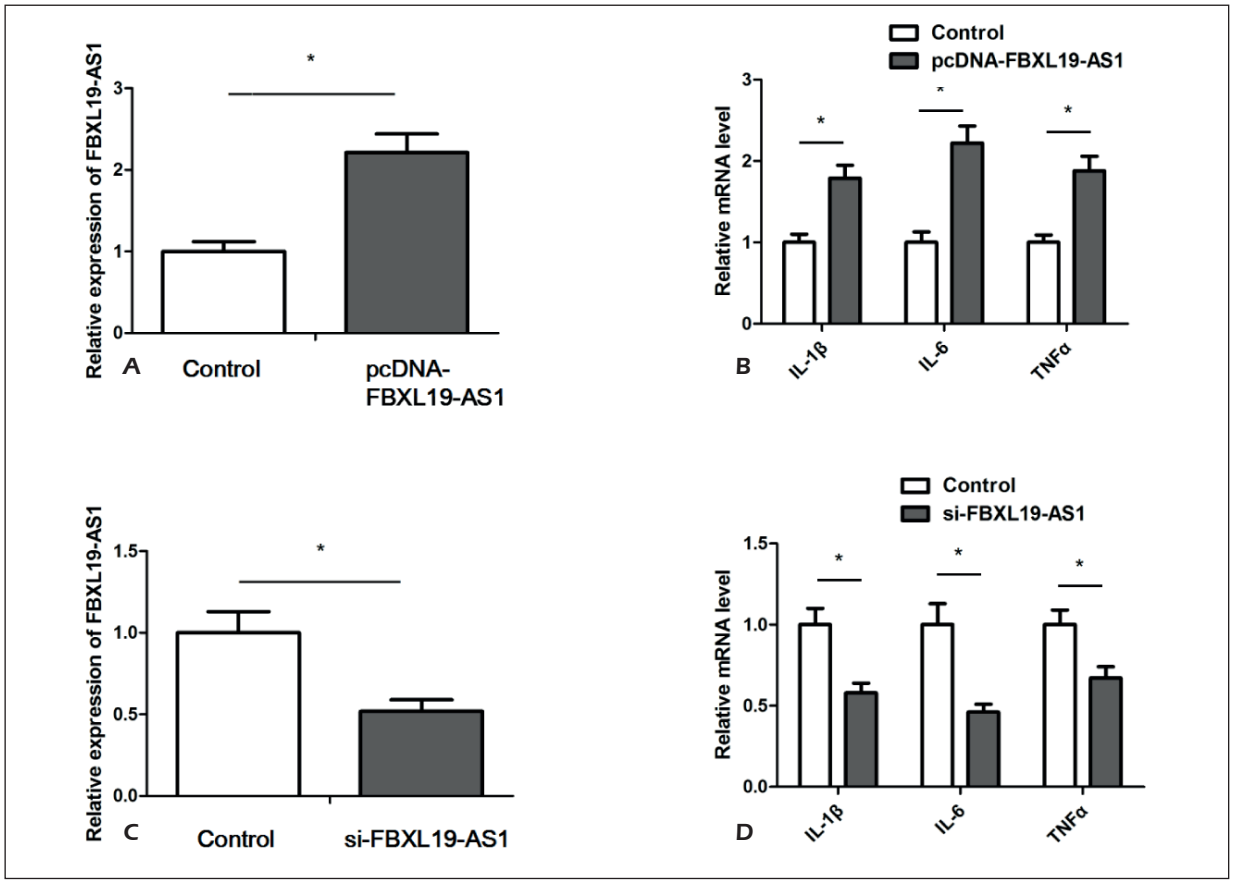

Figure 2. FBXL19-AS1 promoted expressions of IL-1β, IL-6 and TNF-α. **A**, The expression of FBXL19-AS1 in cells was markedly up-regulated after overexpression of FBXL19-AS1. **B**, The expression of FBXL19-AS1 in cells was notably down-regulated after knockdown of FBXL19-AS1. **C**, The results of qRT-PCR manifested that the mRNA expressions of IL-1β, IL-6 and TNF-α remarkably rose after overexpression of FBXL19-AS1 in cells. **D**, The results of qRT-PCR manifested that the mRNA expressions of IL-1β, IL-6 and TNF-α obviously declined after knockdown of FBXL19-AS1 in cells.

in cells (Figure 2C), while the opposite results were obtained after knockdown of FBXL19-AS1 in cells (Figure 2D). The above findings demonstrate that FBXL19-AS1 promotes the expressions of inflammatory factors and induces the inflammatory response in cells, thereby facilitating the occurrence and development of AP.

Discussion

AP is a clinically common acute abdominal disease, which refers to a series of inflammatory responses (self-digestion, hemorrhage and edema) in pancreatic tissues due to multiple causes[12]. The typical clinical manifestations of AP include acute epigastric pain, vomiting and nausea. With the improvement of people's living standards in dietary structure, the morbidity rate of AP is increasing year by year. MAP has a better prognosis and lighter disease burden, but it will easily develop into SAP if not promptly diagnosed and treated, thereby resulting in severe complications, such as peritonitis, sepsis, multiple organ failure, and even death in severe cases[13].

LncRNAs are more than 200 nt in transcript length with no protein coding ability, mainly located in the nucleus or cytoplasm[14]. LncRNAs exerted a wide range of molecular and cellular functions[15] and lncRNAs are involved in many cancers, such as colorectal cancer, bladder cancer, and lung cancer[16-18]. Moreover, it has been found that lncRNA FENDRR is upregulated in the AP cell model, and affects the progression of AP[19]. In this research, the results showed that the serum FBXL19-AS1 level significantly rose in AP patients, which was positively correlated with the predictive scores of AP. Patients with a high expression of FBXL19-AS1 had a higher risk of developing AP and worsening the condition of disease.

With the deepening of research on AP, there has been evidence that systemic inflammatory responses occur in AP. Under the combined action of digestive enzymes and lysosomal enzymes at the initial stage, trypsin is activated, and pancreatic acinar cells are destroyed[20]. The activated trypsin is secreted into the pancreatic interstitium, causing pancreatic self-digestion, and the damaged pancreas can activate local inflammatory cells to secret and release inflammatory mediators (cytokines). In the case of systemic inflammatory responses, cytokines, such as IL-6, IL-8 and TNF-α will be released into the blood circulation[21,22], further enhancing the pro-inflammatory signals. In recent years, the correlation between lncRNAs and immunoregulation in inflammatory diseases has become a research focus. LncRNAs can regulate the body's immune response *via* regulating the protein-protein interaction or DNA-RNA base pairing, thus affecting the differentiation and migration of immune cells and the secretion of inflammatory mediators[23]. In particular, lncRNA NEAT1 is prominently highly expressed in sepsis patients, and it is positively correlated with APACHE II score, serum TNF-α, IL-1β, IL-6 and IL-8, which can raise the risk and worsen the condition of sepsis. Besides, it is also related to poor prognosis and inflammatory factors[24]. The above findings are similar to the results in this study that FBXL19-AS1 could stimulate the expressions of IL-1β, IL-6 and TNF-α, and induce the inflammatory response, thus leading to the occurrence and development of AP.

This study for the first time clarified the relationship between FBXL19-AS1 and acute pancreatitis, which opened a new perspective for understanding the molecular mechanism of acute pancreatitis. Our results are of significance to clarify the pathogenesis of acute pancreatitis and develop novel drug targets.

Conclusions

Summarily, FBXL19-AS1 is positively correlated with the severity of AP. AP patients with a high expression of FBXL19-AS1 are more likely to develop severe AP, which needs to be paid further attention to by clinicians.

Conflict of Interests

The authors declare that they have no conflict of interest.

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