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Expression levels of *RUNX3* and *FGFR2* in peripheral blood of severe acute pancreatitis and their clinical significance

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Purpose: Severe acute pancreatitis (SAP) is a life-threatening inflammatory syndrome of the pancreas. This study aimed to analyze the clinical significance of *runt-associated transcription factor 3* (*RUNX3*) and *fibroblast growth factor receptor 2* (*FGFR2*) expression alterations in SAP.
Methods: This study included 18 SAP patients in Wuzhong People's Hospital from November 2019 to December 2021 and 18 healthy controls. *RUNX3* and *FGFR2* expression levels were determined by RT-quantitative PCR. Correlations between *RUNX3*/*FGFR2* and sex, age, etiology, CRP, procalcitonin, AST, LDH, BUN, Acute Physiology and Chronic Health Evaluation II (APACHE II), Ranson score, Bedside Index for Severity in Acute Pancreatitis (BISAP) score, sequential organ failure assessment (SOFA), and modified computed tomography severity index (MCTSI) score were analyzed. Diagnostic values of *RUNX3* and *FGFR2* in SAP were analyzed using the receiver-operating characteristic curve. The binding of *RUNX3* to *FGFR2* was analyzed by chromatin immunoprecipitation.
Results: *RUNX3* and *FGFR2* were downregulated in peripheral blood of SAP patients. *RUNX3* and *FGFR2* were negatively correlated with CRP, procalcitonin, AST, LDH, BUN, APACHE II score, Ranson score, BISAP score, SOFA score, and MCTSI score. Sensitivity and specificity of *RUNX3* level of <0.9650 for SAP diagnosis were 88.89% and 72.22%, respectively. Sensitivity and specificity of *FGFR2* level of <0.8950 for SAP diagnosis were 66.67% and 83.33%, respectively. *RUNX3* was enriched in the *FGFR2* promoter and was positively correlated with *FGFR2*.
Conclusion: *RUNX3* and *FGFR2* were downregulated in peripheral blood of SAP patients and served as candidate biomarkers for SAP diagnosis. *RUNX3* bound to the *FGFR2* promoter to promote *FGFR2* transcription.
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Key Words: Clinical relevance, Fibroblast growth factor receptor 2, Pancreatitis, Runt-associated transcription factor 3, Serum

INTRODUCTION

Acute pancreatitis (AP) is characterized by local and systemic inflammatory responses and represents a major cause of acute admission to hospital [1]. Severe AP (SAP), accounting for 20% of AP cases, is accompanied by necrosis of the pancreatic or peripancreatic tissues, multiple organ failures, and even high mortality [2]. In clinical trials, therapeutic agents, such as octreotide, antioxidants, pentoxifylline, and lexipafant, have impacts on reversing some clinical indicators [3]. Other drugs, such as ruscogenin exert a protective function in the preclinical stage by reducing proinflammatory cytokine levels [4]. Despite

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that, there is no drug that can consistently prevent the outcome of SAP. In this context, it is significant to identify novel biomarkers to improve the diagnosis and therapeutic outcome of SAP.

Runt-related transcription factor 3 (RUNX3), a member of mammalian Runt-domain transcription factors, plays a role in mediating immunity, inflammation, and cancer, and *RUNX3* deficiency is associated with dysfunction of multiple important organs [5]. For instance, *RUNX3* downregulation disturbs the balance between T helper 1/Th2 cells to induce airway inflammation [6], and dysregulation of *RUNX3* aggravates inflammatory bowel disease via cytokine signaling [7]. Interestingly, *RUNX3* is found to be downregulated in the serum of rats with the progression of SAP and protects against pancreas damage and relevant complications [8]. However, its impact on the severity assessment and diagnosis of SAP remains elusive.

Fibroblast growth factor receptor 2 (FGFR2), an isoform of the FGFR family, emerges as a critical regulator of tumorigenesis and inflammatory responses [9,10]. A former GeneChip analysis has revealed *FGFR2* as an upregulated gene in caerulein-induced pancreatitis [11]. Beyond that, *FGFR2* overexpression alleviates apoptosis and inflammatory responses in caerulein-treated pancreatic duct epithelial cells [12]. *RUNX3* as a transcription factor can bind to the gene promoter to promote the transcription of downstream genes [13], and the JASPAR database (http://jaspar.genereg.net/) further predicted the binding site of *RUNX3* and *FGFR2*, hinting at the regulation of *RUNX3* on *FGFR2* expression. Nevertheless, expression patterns of *RUNX3* and *FGFR2* in peripheral blood of SAP patients, their diagnostic values in SAP, and whether *RUNX3* can regulate *FGFR2* expression in SAP have not been reported before. In light of the aforementioned evidence, we speculated that alterations in *RUNX3* and *FGFR2* are associated with the pathogenesis of SAP and they can serve as candidate biomarkers for the diagnosis of SAP. Principally, our study set out to unveil expression patterns of *RUNX3* and *FGFR2* in peripheral blood of SAP patients, their diagnostic values, and their binding relationship in SAP.

METHODS

Study subjects

In this prospective study, we included 18 SAP patients who received treatment in Wuzhong People's Hospital and Dushu Lake Hospital affiliated to Soochow University from November 2019 to December 2021, with 18 healthy volunteers as the control group. This study was approved by the Academic Ethics Committee of Wuzhong People's Hospital (No. 20170235). All participants were informed of the objective of this study and signed the informed consent before acquisition of clinical samples.

Inclusion and exclusion criteria

The inclusion criteria were as follows [14]: (1) at the age of 18 years old or older; (2) AP diagnosis conformed to at least 2 of 3 of the following criteria: (a) characteristic abdominal pain; (b) serum amylase activity at least 3 times higher than the normal upper limit; and (c) characteristic findings from abdominal imaging; (3) SAP is diagnosed as AP with organ failure lasting more than 48 hours; and (4) admission to the hospital within 48 hours of the disease onset. The exclusion criteria were as follows: (1) a history of chronic pancreatitis; (2) during pregnancy or lactation period; (3) blood collection was insufficient for analysis; (4) symptoms of abdominal pain lasted more than 48 hours before admission to hospital; and (5) receiving the immunosuppressive therapy.

Collection of samples and data

The following data of included patients and control population were collected: sex, age, etiology (including alcohol, biliary, hypertriglyceridemia, others), CRP, procalcitonin (PCT), AST, LDH, BUN, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Ranson score, Bedside Index for Severity in Acute Pancreatitis (BISAP) score, sequential organ failure assessment (SOFA), and modified computed tomography severity index (MCTSI) score.

Assays of serum indexes enzyme-linked immunosorbent assay

During admission to the hospital, 3 mL of fasting peripheral blood was collected from each subject and preserved in vacuum tubes without anticoagulant or anticoagulant tubes containing ethylenediamine tetraacetic acid-K2. Blood samples were centrifuged at 1,450 × *g* for 10 minutes to separate the serum. The upper serum was collected and preserved in a refrigerator at –80 °C. Then, levels of CRP (ab260058, Abcam), PCT (ab221828, Abcam), and AST (ab263881, Abcam) were determined using enzyme-linked immunosorbent assay kits. According to the protocols, LDH level was determined using the LDH assay kit (colorimetric; ab102526, Abcam), and BUN level was measured using the BUN assay kit (urease method; Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Pancreatitis scoring systems

According to previous studies and existing scoring criteria [15], the severity of AP was evaluated using the APACHE II scoring system. The total score of APACHE II was 71 and a score of ≥8 was used as the standard to confirm SAP. The higher the APACHE II score, the severer the AP.

Referring to a previous study [16], the severity of AP was evaluated using the Ranson scoring system. This scoring system

included 5 clinical indicators at admission and 6 indicators within 48 hours after admission. Each indicator accounted for 1 score, with a total of 11 scores, and a score of ≥3 was used as the standard to confirm SAP. Indicators at admission included: age, >55 years; blood glucose, >11.1 mmoL/L; serum AST, >250 U/L; serum LDH, >350 U/L; and the number of leukocytes, >16 × 10^9/L. Indicators within 48 hours after admission included: serum calcium concentration, <8 mg/dL/L; arterial PaO_2, <60 mmHg; base deficit, >4 mmol/L; increase in serum BUN, >5 mg/dL; reduction in hematocrit, >10%; and loss of body fluid, >6 L. The higher the Ranson score, the severer the AP.

Referring to a previous study [17], the severity of AP was evaluated using the BISAP scoring system. The BISAP scoring system included the following variables: BUN level, >25 mg/dL; impaired mental status; systemic inflammatory response syndrome; age, >60 years; and presence of pleural effusion. The total score of BISAP was 5 and a score of ≥3 was used as the standard to confirm SAP. The higher the BISAP score, the severer the AP.

Referring to a previous study [18], the severity of AP was evaluated using the SOFA scoring system. This scoring system included 6 standards to reflect the functions of organ systems (respiratory system, hematologic system, liver system, cardiovascular system, nervous system, and kidney system). Each item accounted for 0–4 scores, with a total of 24 scores. The higher the SOFA score, the severer the AP.

Referring to a previous study [19], the severity of AP was evaluated using the MCTSI scoring system. Its scoring criteria were as follows: (1) pancreatic inflammation: 0, normal pancreas; 2, intrinsic pancreatic abnormalities with peripancreatic inflammatory changes; and 4, pancreatic or peripancreatic fluid collection or peripancreatic fat necrosis; (2) pancreatic necrosis: 0, no necrosis; 2, <30% necrosis; and 4, ≥30% necrosis; (3) extrapancreatic complications: 2, pleural effusion, ascites, vascular complications (venous thrombosis, arterial hemorrhage, pseudoaneurysm), parenchymal complication (infarction, hemorrhage, subcapsular fluid collection), or gastrointestinal involvement (inflammation, perforation, intraluminal fluid collection). The MCTSI score of ≥4 was used as the standard to confirm SAP. The higher the MCTSI score, the severer the AP.

Real-time quantitative PCR

The total RNA was extracted from the serum using the TRIzol reagent (Life Technologies) following the manufacturer's instructions. The total RNA was reverse-transcribed into the complementary DNA using the Prime Script RT Master Mix (Takara). RT-quantitative PCR (qPCR) was performed on the Step One Plus Real-Time PCR system (Applied Biosystems) using the Fast Start Universal SYBR Green Master mix. With glyceraldehyde 3-phosphate dehydrogenase as the endogenous reference, the relative amount of gene expression was quantified using the $2^{-\Delta\Delta Ct}$ method. Primers used for qPCR are shown in Table 1.

Table 1. Quantitative PCR primers

Gene	Sequence (5′-3′)
RUNX3	Forward primer: ATTCATTCATTCCCCGTGGC
	Reverse primer: AAGCGAAGGTCGTTGAACCT
FGFR2	Forward primer: CCTGCGGAGACAGGTAACAG
	Reverse primer: CAGTTCGTTGGTGCAGTTGG
GAPDH	Forward primer: GTTAGGAAAGCCTGCCGGTG
	Reverse primer: AGCATCGCCCCACTTGATTT

Chromatin immunoprecipitation assay

The binding site of *RUNX3* to the *FGFR2* promoter was predicted on the JASPAR database [20]. To testify the binding of *RUNX3* to the *FGFR2* promoter, the chromatin immunoprecipitation (ChIP) assay was performed using the Pierce Magnetic ChIP kit (Thermo Fisher Scientific). In brief, 293T cells were crosslinked with 1% formaldehyde for 10 minutes at room temperature, followed by nuclear separation and ultrasonic processing in a lysis buffer. Next, chromatins were incubated with the antibody against *RUNX3* (MA5-17169, Thermo Fisher Scientific) or immunoglobulin G (B-2763, Thermo Fisher Scientific) for immunoprecipitation. Eventually, the immunoprecipitated DNA-protein complex was extracted and purified using the fragment DNA kit (Intron Biotechnology). Primer sequences of the *FGFR2* promoter region: forward 5'-ACAGAACCCCCGTGAAGATG-3'; reverse 5'-GGAGATGGGTGGG CAAGAAT-3'.

Data analysis

Data statistical analysis and graphing were conducted using IBM SPSS Statistics ver. 21.0 (IBM Corp.) and GraphPad Prism 8.0 software (GraphPad Software Inc.). Data were classified into enumeration and measurement data. Enumeration data were expressed as case numbers and measurement data were expressed as mean ± standard deviation. Pairwise comparisons of enumeration data were analyzed using the chi-square test and pairwise comparisons of measurement data were analyzed using the independent-sample t-test. The diagnostic values of *RUNX3* and *FGFR2* in SAP were analyzed using the receiver-operating characteristic (ROC) curve. Correlations between *RUNX3*/*FGFR2* expression in peripheral blood of SAP patients and clinicopathological characteristics of SAP patients were analyzed using Pearson correlation analysis. P-values were obtained from two-sided tests and a P-value of <0.05 was indicative of statistical significance.

RESULTS

Clinical data of the included population

This study included 18 SAP patients and 18 healthy volunteers. We collected relevant clinical data from all study subjects and observed that among these included people, there was no significant difference in age between SAP patients and the controls ($P > 0.05$). Levels of CRP, PCT, LDH, AST, and BUN in SAP patients were significantly higher than those in the controls ($P < 0.05$) (Table 2).

RUNX3 is downregulated in peripheral blood of severe acute pancreatitis patients and has high diagnostic values

RUNX3 has been noted to be downregulated in the SAP rat model [8], but its expression pattern in peripheral blood of SAP

Table 2. Clinical baseline characteristics

Variable	Control group	SAP group	P-value
No. of patients	18	18	
Sex			0.735[a]
Male	11	10	
Female	7	8	
Age (yr)			0.738[a]
≤55	9	8	
>55	9	10	
Etiology			
Alcohol	-	4	-
Biliary	-	7	-
Hypertriglyceridemia	-	4	-
Others	-	3	-
CRP (mg/L)	14.39 ± 3.26	145.08 ± 27.23	<0.001[b]
PCT (μg/L)	0.23 ± 0.06	3.38 ± 0.91	<0.001[b]
LDH (U/L)	113.58 ± 44.50	528.50 ± 137.86	<0.001[b]
AST (U/L)	30.67 ± 5.00	102.39 ± 25.12	<0.001[b]
BUN (mmol/L)	4.13 ± 0.99	6.33 ± 1.01	<0.001[b]
APACHE II score	-	10.33 ± 1.53	-
Ranson score	-	3.61 ± 0.92	-
BISAP score	-	3.39 ± 0.92	-
SOFA score	-	5.67 ± 1.14	-
MCTSI score	-	4.78 ± 1.11	-

Values are presented as number or mean ± standard deviation.
SAP, severe acute pancreatitis; PCT, procalcitonin; APACHE II, Acute Physiology and Chronic Health Evaluation II; BISAP, Bedside Index for Severity in Acute Pancreatitis; SOFA, sequential organ failure assessment; MCTSI, modified computed tomography severity index.
For data analysis, [a]the Fisher test and [b]the independent-sample t-test was used.

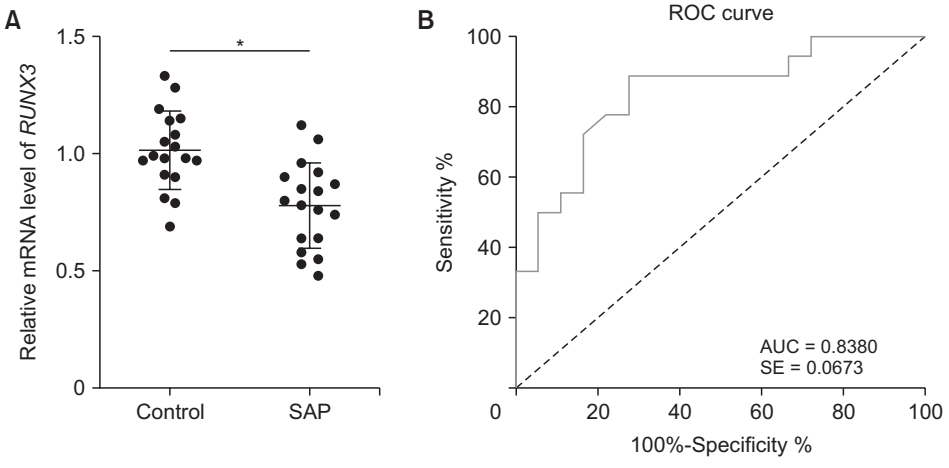

Fig. 1. *RUNX3* is downregulated in peripheral blood of SAP patients and has high diagnostic values. (A) *RUNX3* levels in peripheral blood of SAP patients and the control group were determined by RT-quantitative PCR. (B) ROC curve based on the diagnostic value of *RUNX3* in SAP. Data in panel A were analyzed using the independent-sample t-test. SAP, severe acute pancreatitis; ROC, receiver-operating characteristic; AUC, the area under the curve; SE, standard error.

patients remains unknown. RT-qPCR revealed that compared with the control group, *RUNX3* was weakly expressed in SAP patients ($P < 0.05$) (Fig. 1A). Furthermore, we plotted an ROC curve based on the diagnostic value of *RUNX3* in SAP (Fig. 1B), which revealed the area under the curve (AUC) of 0.8380 and a cutoff point of 0.9650 (sensitivity, 88.89%; specificity, 72.22%). The results indicated that *RUNX3* level in peripheral blood of <0.9650 can help the diagnosis of SAP.

RUNX3 expression is correlated with clinicopathological features of severe acute pancreatitis patients

To explore the correlation between *RUNX3* expression and clinicopathological characteristics of SAP patients, the median *RUNX3* expression was set as the critical threshold [21] to classify 18 SAP patients into the high *RUNX3* expression group and the low *RUNX3* expression group. Our results showed that *RUNX3* expression had no significant correlation with sex, age, and etiology ($P > 0.05$) but had correlations with CRP, PCT, LDH, AST, BUN, APACHE II score, Ranson score, BISAP score, SOFA score, and MCTSI score ($P < 0.05$) (Table 3). In addition, negative correlations between *RUNX3* expression in peripheral blood and CRP, PCT, LDH, AST, BUN, APACHE II score, Ranson score, BISAP score, SOFA score, and MCTSI score were found in 18 SAP patients (Fig. 2A–K).

FGFR2 is downregulated in peripheral blood of severe acute pancreatitis patients and has high diagnostic values

FGFR2 has been demonstrated to be downregulated in AP cell models [12], but its expression pattern in peripheral blood of SAP patients remains unknown. RT-qPCR revealed that compared with the control group, *FGFR2* was poorly expressed in the peripheral blood of SAP patients ($P < 0.05$) (Fig. 3A). We further plotted an ROC curve based on the diagnostic value of *FGFR2* in SAP (Fig. 3B), which revealed the AUC of 0.7438 and a cutoff point of 0.8950 (sensitivity, 66.67%; specificity, 83.33%). The results indicated that *FGFR2* level in peripheral blood < 0.8950 can help the diagnosis of SAP.

FGFR2 expression is correlated with clinicopathological features of severe acute pancreatitis patients

To explore the correlations between *FGFR2* expression and clinicopathological characteristics of SAP patients, the median *FGFR2* expression was set as the critical threshold to classify 18

Table 3. Correlations between *RUNX3* expression and clinicopathological characteristics of SAP patients

Variable	*RUNX3*		P-value
	Low expression (n = 9)	High expression (n = 9)	
Sex			0.637[a]
Male	4	6	
Female	5	3	
Age (yr)			0.153[a]
≤55	2	6	
>55	7	3	
Etiology			0.637[a]
Alcohol	3	1	
Biliary	4	3	
Hypertriglyceridemia	1	3	
Others	1	2	
CRP (mg/L)	159.39 ± 22.13	130.76 ± 25.00	0.020[b]
PCT (μg/L)	3.90 ± 0.90	2.87 ± 0.61	0.011[b]
LDH (U/L)	630.23 ± 96.49	426.77 ± 88.27	<0.001[b]
AST (U/L)	122.82 ± 17.34	81.97 ± 10.05	<0.001[b]
BUN (mmol/L)	6.99 ± 0.80	5.68 ± 0.76	0.003[b]
APACHE II score	11.22 ± 1.39	9.44 ± 1.13	0.009[b]
Ranson score	4.11 ± 0.78	3.11 ± 0.78	0.015[b]
BISAP score	3.89 ± 0.78	2.89 ± 0.78	0.015[b]
SOFA score	6.22 ± 1.09	5.11 ± 0.93	0.034[b]
MCTSI score	5.44 ± 1.01	4.11 ± 0.78	0.007[b]

Values are presented as number or mean ± standard deviation.
PCT, procalcitonin; APACHE II, Acute Physiology and Chronic Health Evaluation II; BISAP, Bedside Index for Severity in Acute Pancreatitis; SOFA, sequential organ failure assessment; MCTSI, modified computed tomography severity index.
For data analysis, [a]the Fisher test and [b]the independent-sample t-test was used.

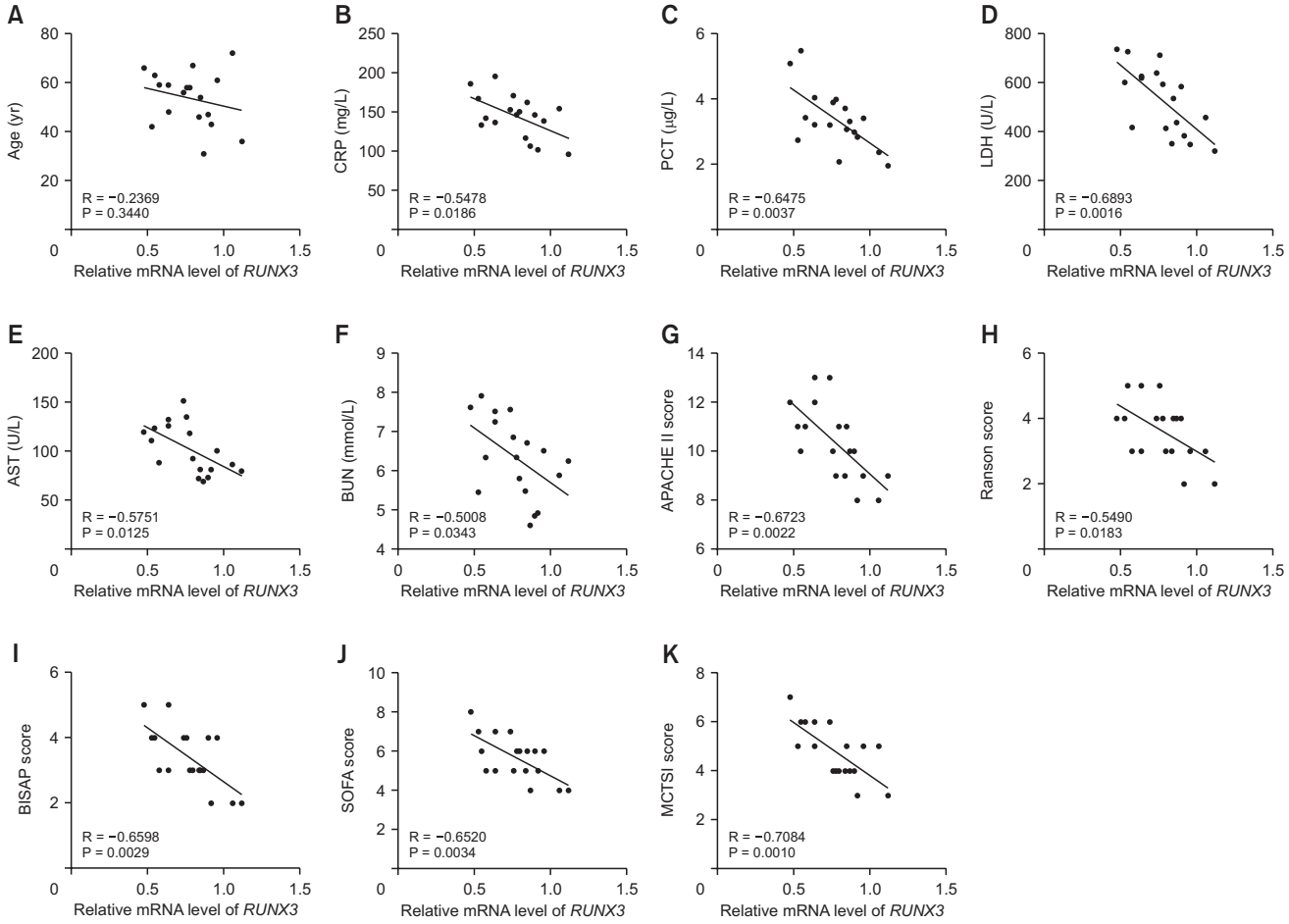

Fig. 2. *RUNX3* expression is correlated with clinicopathological characteristics of SAP patients. (A–K) Correlations between *RUNX3* expression in peripheral blood and CRP, PCT, LDH, AST, BUN, APACHE II score, Ranson score, BISAP score, SOFA score, and MCTSI score in 18 SAP patients were analyzed using Pearson correlation analysis. SAP, severe acute pancreatitis; PCT, procalcitonin; APACHE II, Acute Physiology and Chronic Health Evaluation II; BISAP, Bedside Index for Severity in Acute Pancreatitis; SOFA, sequential organ failure assessment; MCTSI, modified computed tomography severity index.

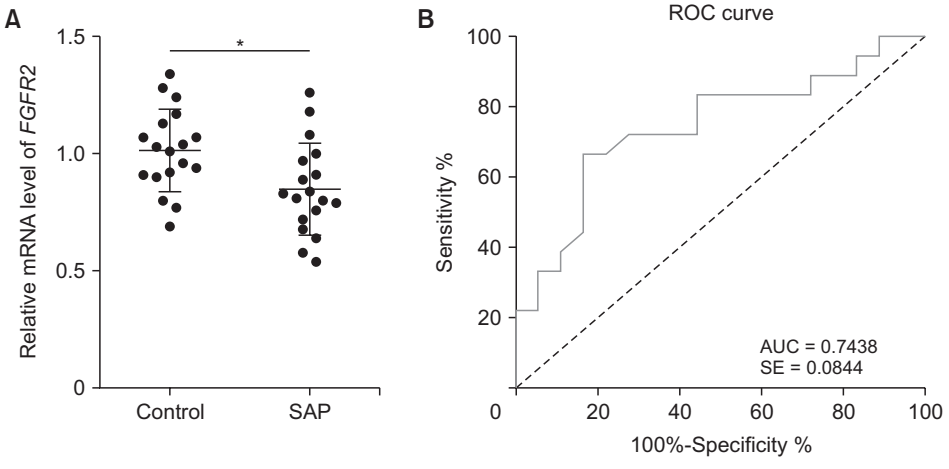

Fig. 3. *FGFR2* is downregulated in peripheral blood of SAP patients and has high diagnostic values. (A) *FGFR2* levels in peripheral blood of SAP patients and the control group were determined by RT-quantitative PCR. (B) ROC curve based on the diagnostic value of *FGFR2* in SAP. Data in panel A were analyzed using the independent-sample t-test. SAP, severe acute pancreatitis; ROC, receiver-operating characteristic; AUC, the area under the curve; SE, standard error. *P < 0.05.

Table 4. Correlation between *FGFR2* expression and clinicopathological characteristics of SAP patients

Variable	*FGFR2*		P-value
	Low expression (n = 9)	High expression (n = 9)	
Sex			0.637[a]
Male	6	4	
Female	3	5	
Age (yr)			0.637[a]
≤55	3	5	
>55	6	4	
Etiology			0.499[a]
Alcohol	3	1	
Biliary	2	5	
Hypertriglyceridemia	2	2	
Others	2	1	
CRP (mg/L)	158.42 ± 22.31	131.73 ± 26.03	0.033[b]
PCT (μg/L)	3.83 ± 0.91	2.94 ± 0.72	0.036[b]
LDH (U/L)	614.35 ± 119.93	442.64 ± 97.05	<0.001[b]
AST (U/L)	115.90 ± 25.66	88.89 ± 16.47	<0.001[b]
BUN (mmol/L)	6.98 ± 0.92	5.68 ± 0.62	0.003[b]
APACHE II score	11.11± 1.45	9.56 ± 1.24	0.026[b]
Ranson score	4.11 ± 0.78	3.11 ± 0.78	0.015[b]
BISAP score	4.00 ± 0.71	2.78 ± 0.67	0.002[b]
SOFA score	6.22 ± 0.97	5.11 ± 1.05	0.034[b]
MCTSI score	5.33 ± 1.00	4.22 ± 0.97	0.029[b]

Values are presented as number or mean ± standard deviation.
PCT, procalcitonin; APACHE II, Acute Physiology and Chronic Health Evaluation II; BISAP, Bedside Index for Severity in Acute Pancreatitis; SOFA, sequential organ failure assessment; MCTSI, modified computed tomography severity index.
For data analysis, [a]the Fisher test and [b]the independent-sample t-test was used.

SAP patients as the high *FGFR2* expression group and the low *FGFR2* expression group. Our results uncovered that there was no significant correlation between *FGFR2* expression and sex, age, and etiology ($P > 0.05$) but there were correlations between *FGFR2* expression and CRP, PCT, LDH, AST, BUN, APACHE II score, Ranson score, BISAP score, SOFA score, and MCTSI score ($P < 0.05$) (Table 4). Additionally, *FGFR2* expression in peripheral blood was negatively correlated with CRP, PCT, LDH, AST, BUN, APACHE II score, Ranson score, BISAP score, SOFA score, and MCTSI score in 18 SAP patients ($P < 0.05$) (Fig. 4).

RUNX3 expression is positively correlated with *FGFR2* expression in peripheral blood of severe acute pancreatitis patients

As indicated by a previous study, *RUNX3* is a transcription activator of its downstream genes [13]. Through the JASPAR database, *RUNX3* was predicted to bind to the *FGFR2* promoter (Fig. 5A). The ChIP assay revealed the enrichment of *RUNX3* on the *FGFR2* promoter ($P < 0.05$) (Fig. 5B). Also, a positive correlation between *RUNX3* expression and *FGFR2* expression was found in the peripheral blood of 18 SAP patients ($P < 0.05$) (Fig. 5C). Above all, *RUNX3* was enriched on the *FGFR2* promoter and *RUNX3* expression was positively correlated with *FGFR2* expression.

DISCUSSION

SAP is a life-threatening inflammatory syndrome with a series of complications. SAP is complex in its pathogenesis and lacks effective therapeutic options [3]. Identification of molecular biomarkers is beneficial for the diagnosis and treatment of SAP. In this prospective study, we determined expression patterns of *RUNX3* and *FGFR2* in peripheral blood of SAP patients and analyzed correlations between *RUNX3*/*FGFR2* expression and clinicopathologic features of SAP. Our findings uncovered that *RUNX3* and *FGFR2* were downregulated in peripheral blood of SAP patients and correlated with clinicopathological features of SAP, and *RUNX3* was enriched in the *FGFR2* promoter and promoted *FGFR2* transcription.

Serum levels of CRP and PCT may be used as indicators of liver injury in AP patients [22]. Serum PCT, CRP, LDH, AST, and BUN also contribute to the diagnosis and prognosis of SAP [23,24]. In our cohorts, levels of CRP, PCT, LDH, AST, and BUN were found to be significantly higher in SAP patients, nominating them vital indicators of SAP diagnosis. Accumulating evidence has confirmed that a myriad of

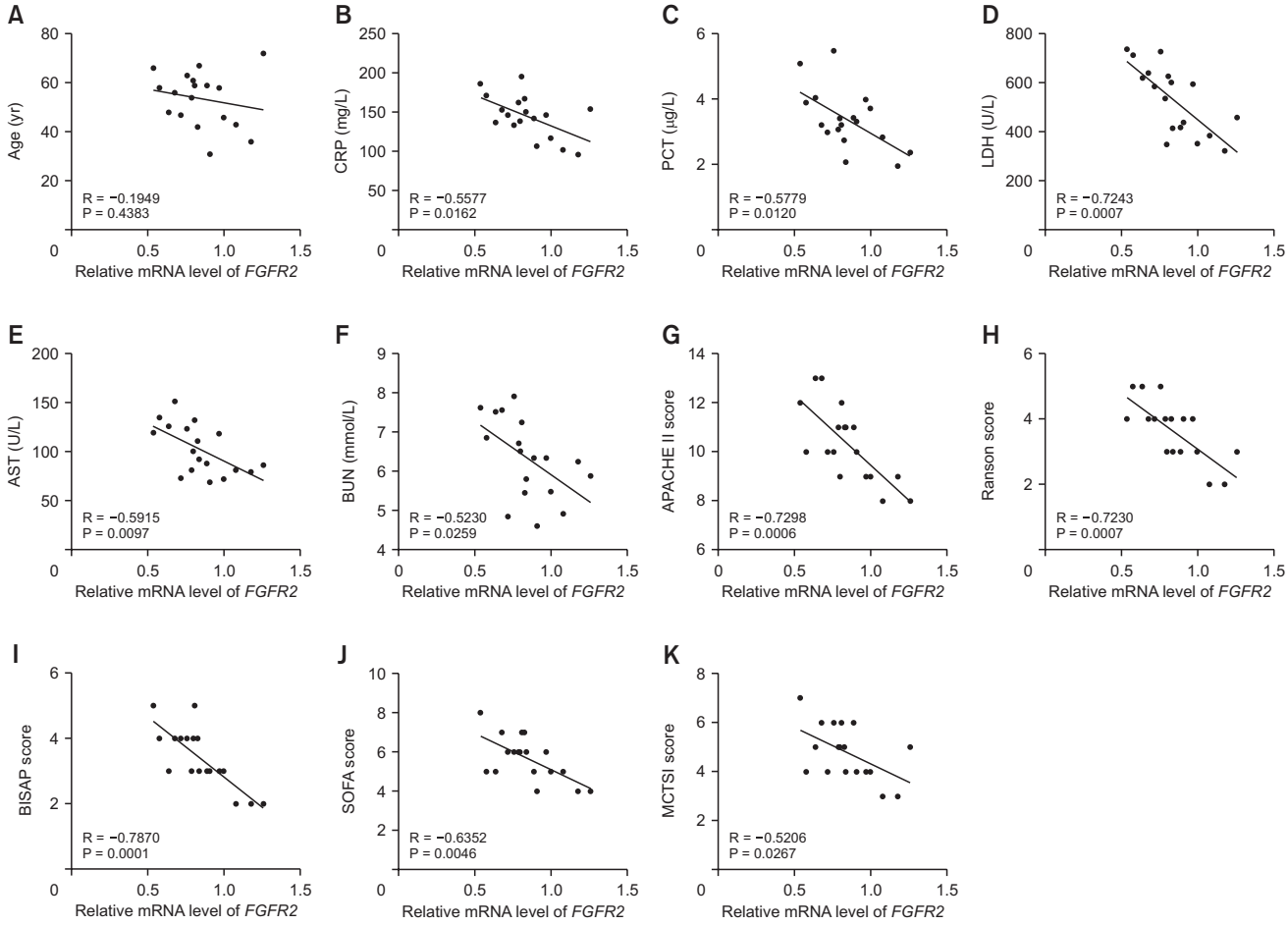

Fig. 4. *FGFR2* expression is correlated with clinicopathological characteristics of SAP patients. (A–K) Correlations between *FGFR2* expression in peripheral blood and CRP, PCT, LDH, AST, BUN, APACHE II score, Ranson score, BISAP score, SOFA score, and MCTSI score in 18 SAP patients were analyzed using Pearson correlation analysis. SAP, severe acute pancreatitis; PCT, procalcitonin; APACHE II, Acute Physiology and Chronic Health Evaluation II; BISAP, Bedside Index for Severity in Acute Pancreatitis; SOFA, sequential organ failure assessment; MCTSI, modified computed tomography severity index.

transcription factors act as positive or negative regulators of pancreatitis, including *RUNX3* [8]. *RUNX3* is also involved in the immune system and inflammatory pathways [25]. *RUNX3* expression is associated with pancreas tumorigenesis [26]. Decreased *RUNX3* levels were found in peripheral blood of SAP patients and *RUNX3* level was negatively correlated with CRP, PCT, LDH, AST, and BUN and severity scores in SAP patients. The ROC curve revealed that the AUC value of *RUNX3* was 0.8380, the cutoff value of *RUNX3* was 0.9650, and the sensitivity and specificity of *RUNX3* serving as a peripheral blood biomarker of SAP were 88.89% and 72.22%, respectively, suggestive of high diagnostic value of *RUNX3* in SAP. In previous studies, *RUNX3* overexpression alleviated pancreas damage and lung injury in SAP rats by repressing the Janus kinase 2/signal transducer and activator of transcription 3 pathway [8]. In addition, loss of *RUNX3* contributes to the inflammatory reaction as a precancerous state in multiple organs [5]. For example, *RUNX3*-deficient lymphocytes triggers colitis and then induces the formation of colon tumors [27]. In non-tumor inflammatory status, *RUNX3* can be directly downregulated by environmental risks or transcriptionally repressed by the upstream signaling. For instance, PM2.5 exposure downregulates *RUNX3* to induce airway inflammation [6]. Altogether, our findings suggested that *RUNX3* was a candidate biomarker for SAP diagnosis.

FGF-FGFR signaling is a major mechanism for the development of inflammation [28]. Activation of FGF10/*FGFR2* restrains the release of proinflammatory cytokines after spinal cord injury [29]. On another note, our data revealed that *FGFR2* had similar characteristics of *RUNX3* in SAP patients. *FGFR2* were downregulated in the peripheral blood of SAP patients and were negatively correlated with CRP, PCT, LDH, AST, and BUN and severity scores. The ROC curve showed that an *FGFR2* level of <0.8950 was beneficial for SAP diagnosis, with sensitivity of 66.67% and specificity of 83.33%. As known before, the maternally expressed gene 3/miR-195-5p/*FGFR2* axis mitigates

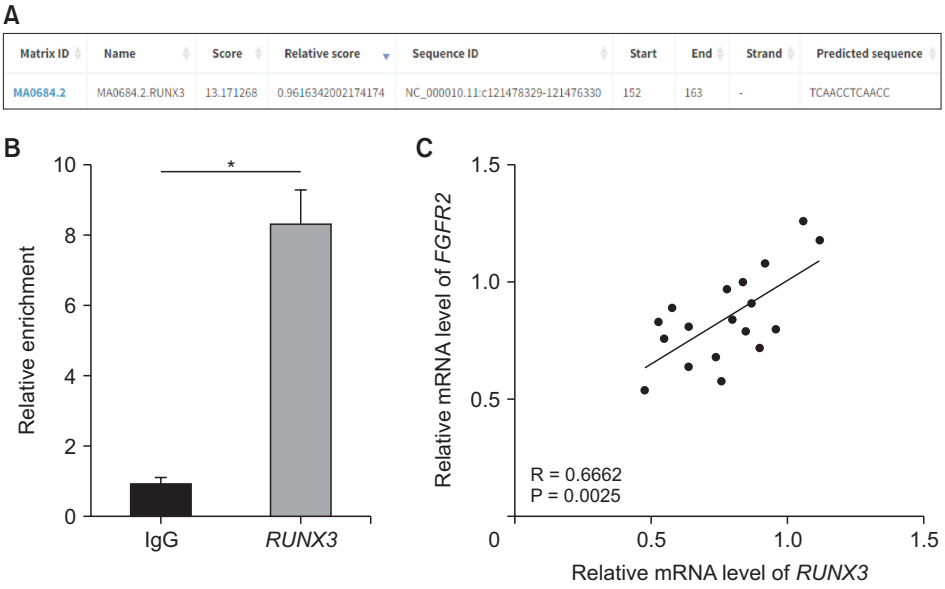

Fig. 5. *RUNX3* expression is positively correlated with *FGFR2* expression in peripheral blood of SAP patients. (A) The binding of *RUNX3* to the *FGFR2* promoter was predicted on the JASPAR database (http://jaspar.genereg.net/). (B) The enrichment of *RUNX3* on the *FGFR2* promoter was analyzed by the ChIP assay. (C) Correlation between *RUNX3* expression and *FGFR2* expression in peripheral blood of 18 SAP patients was analyzed by Pearson correlation analysis. Cell experiments were performed 3 times independently. Data in panel B were expressed as mean ± standard deviation and analyzed using the t-test. IgG, immunoglobulin G; SAP, severe acute pancreatitis; ChIP, chromatin immunoprecipitation. *P < 0.05.

inflammatory injury in caerulein-induced pancreatic cells by inactivating nuclear factor-kappa beta [12]. Likewise, blocking the FGF10/*FGFR2b* upregulates the release of pro-inflammatory cytokines, such as tumor necrosis factor alpha, interleukin (IL) 6, and IL-8 in orbital fibroblasts [30]. Furthermore, the JASPAR database and the ChIP assay confirmed the binding relationship between *RUNX3* and the *FGFR2* promoter, and Pearson correlation analysis revealed a positive correlation between *RUNX3* expression and *FGFR2* expression in peripheral blood of SAP patients, suggesting that *RUNX3* may bind to the *FGFR2* promoter to upregulate *FGFR2* transcription and then play a role in SAP. Collectively, our findings made it plausible that *FGFR2* was a candidate biomarker for SAP diagnosis and *RUNX3* may upregulate *FGFR2* transcription in SAP.

In summary, our study initially demonstrated the diagnostic values of *RUNX3* and *FGFR2* in SAP and the binding relationship between them, providing a novel entry point for the clinical disease judgment and novel therapeutic targets for SAP treatment. However, our study is limited to a small volume of clinical samples, and whether *RUNX3* can regulate *FGFR2* to play a role in SAP progression was not validated through cell and animal experiments. Besides, we did not systematically analyze the correlation between hospital time/other clinical parameters and *RUNX3* and *FGFR2* expression. More studies with large sample volume, multiple centers, and cell and animal experiments are needed to confirm the diagnostic values of *RUNX3* and *FGFR2*, analyze the role of the *RUNX3*/*FGFR2* axis in SAP, and explore the upstream factors of the *RUNX3*/*FGFR2* axis in SAP.

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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