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Effects of IFNG-AS1 and ANRIL on intestinal epithelial cells and their relationship with colitis

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Abstract. – OBJECTIVE: To explore the effects of IFNG-AS1 and ANRIL on intestinal epithelial cells and their relationship with colitis.

PATIENTS AND METHODS: From May 2017 to May 2019, 118 colitis patients admitted to our hospital were selected as the research group (RG), and 124 healthy controls were selected as the control group (CG). In addition, the normal intestinal epithelial cells HIEC and HIEC-6 were purchased to detect the IFNG-AS1 and ANRIL in the peripheral blood of patients in the two groups, and the effects of IFNG-AS1 and ANRIL on the intestinal epithelial cells were analyzed.

RESULTS: IFNG-AS1 and ANRIL were highly expressed in colitis ($p<0.050$), and their combined detection had good diagnostic value for the occurrence of colitis and complications ($p<0.050$). In intestinal epithelial cells transfected with IFNG-AS1 and ANRIL, it was found that inhibition of IFNG-AS1 and ANRIL remarkably increased the proliferation and decreased the apoptosis of intestinal epithelial cells ($p<0.050$).

CONCLUSIONS: IFNG-AS1 and ANRIL are highly expressed in colitis, and inhibiting their expression can promote the proliferation of intestinal epithelial cells and reduce apoptosis, which may be potential therapeutic targets for Crohn's colitis in the future.

Key Words:
IFNG-AS1, ANRIL, Colitis, Intestinal epithelial cells, Apoptosis.

Introduction

Colitis is an inflammatory process that is confined to the colonic mucosa and submucosa, which is mostly found in the sigmoid colon and rectum, and can also extend to lower colon[1]. It is mainly divided into ulcerative colitis or ischemic colitis, among which the former accounts for the most cases[2]. According to statistics, the incidence of colitis in clinical practice is about 1.1-14.9/100,000, and increasing studies in recent years show that the incidence is on the rise[3,4]. With a high incidence at all ages, the pathological process of colitis is long, and is usually accompanied with repeated attacks[5]. Irrespective of generally abdominal pain and diarrhea, there are no special clinical symptoms in the early stage of colitis[6]. Worse still, due to the lack of medical and health knowledge, patients may easily ignore or misjudge the onset of the disease, missing the best treatment period[7]. At present, the pathogenesis of colitis is not yet clear. Clinically, it is considered to be an autoimmune disease, which is related to the activity of immunoglobulin and immune cells[8]. In recent years, the focus of researchers at home and abroad have gradually shifted to genetic changes. Among them, long non-coding RNAs (lncRNAs) are non-coding RNAs with a length of more than 200 bp that have been proved to play an essential part in many life activities, such as dose compensation effect, epigenetic regulation, cell cycle regulation and cell differentiation regulation, and is closely correlated with many tumor diseases[9]. Of these, lncRNA IFNG-AS1 has been identified in previous studies to be bound up with inflammatory factors and exert carcinogenicity through interaction with ESRP2[10,11]. While lncRNA ANRIL is a pro-inflammatory gene that mediates NF-κB to play a role in inflammation-related coronary artery disease[12,13]. The current research on colitis between the two is not yet clear, nor is it known whether the two have an impact on the pathogenesis of colitis. We suggest that IFNG-AS1 and ANRIL, as lncRNAs that have been

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shown to be associated with immune diseases, are also significantly associated with the development of colitis. In this regard, we detected the value of IFNG-AS1 and ANRIL in colitis through experimental analysis and explored their effects on intestinal epithelial cells, so as to provide reliable and accurate references for future clinical diagnosis and treatment of colitis.

Patients and Methods

General Information

Totally 118 patients with colitis admitted to our hospital from May 2017 to May 2019 were selected as the research group (RG), and 124 healthy controls during the same period were selected as the control group (CG). Having been approved by the Medical Ethics Committee of our hospital, together with the written informed consent obtained from all the enrolled participants, this study was thus carried out.

Inclusion and Exclusion Criteria

Inclusion criteria: patients who met the clinical manifestations of colitis and were diagnosed with Crohn's colitis by X-ray, colonoscopy and pathological biopsy in our hospital, and those agreed to participate and cooperate with the study with complete clinicopathological data. Exclusion criteria: patients with multiple autoimmune defects, cardio-cerebrovascular diseases, mental disorders or infectious diseases; patients with drug allergy; patients in lactation; patients who had received antibiotic treatment 3 months before operation; patients who died in the course of treatment and rehabilitation; transferred patients.

Cell Source

Purchased from American Type Culture Collection ATCC (Manassas, VA, USA), the normal intestinal epithelial cells HIEC and HIEC-6 were cultured in 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin mixture Dulbecco's Modified Eagle's Medium(DMEM) in an incubator at 37°C with 5% CO_2.

Detection Methods

Polymerase Chain Reaction (PCR)

EasyPure miRNA Kit was employed to extract the total RNA from the collected blood samples, and then the purity, concentration and integrity were detected by UV spectrophotometer and agarose gel electrophoresis. Reverse transcription of RNA into cDNA was performed using 2× TS miRNA Reaction Mix in the TransScript Green miRNA Two-Step qRT-PCR SuperMix kit, and the specific procedures were followed in accordance with the manufacturer's kit instructions. Then came the PCR amplification, with the reaction system as follows: cDNA: 1 μL, upstream and downstream primers: 0.4 μL each, 2×TransTaq® Tip Green qPCR SuperMix: 10 μL, Passive Reference Dye (50×): 0.4 μL, and finally added ddH_2O to achieve 20 μL. PCR reaction conditions: pre-denaturation: 94°C for 30 s, denaturation: 94°C for 5 s, annealing: 60°C for 15 s and then extension for 10 s, totaling 40 cycles. Three replicate wells were set for each sample and the experiment was performed a total of three times. In this study, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference, and $2^{-\Delta\Delta ct}$ was responsible for data analysis (Table I).

Cell Transfection

IFNG-AS1 and ANRIL inhibitory plasmids (IFNG-AS1-inhibit, ANRIL-inhibit), overexpression plasmids (IFNG-AS1-mimics, ANRIL-mimics), and blank control (Blank) were established respectively. The established drug resistant cell lines were transferred to 24-well plates, and then transfected with 100 nM overexpression, inhibition and blank control 48 hours later by means of Lipofectamine 2000 kit (Invitrogen, Carlsbad, CA, USA) in strict accordance with the kit instructions.

Western Blot

The cultured cells were lysed by radioimmunoprecipitation assay buffer (RIPA) buffer (Thermo Fisher Scientific, Waltham, MA, USA) and its protein concentration was detected by

Table I. Primer sequences.

Genes	Upstream (5 '-3')	Downstream (5 '-3')
IFNG-AS1	GCTGATGATGGTGGCAATCT	TTAGCAGTTGGTGGGCTTCT
ANRIL	GCG CCG GAC TAG GAC TAT TT	GCC AGG ACG GAG ATC AGA TG
GAPDH	CACGAAACTACCTTCAACTCC	CATACTCCTGCTTGCTGATC

bicinchoninic acid (BAC) kit (Thermo Fisher Scientific, Waltham, MA, USA) before it was adjusted to 4 μg/μL. Having separated by 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the membrane was transferred to a 0.22 μm polyvinylidene difluoride (PVDF) membrane, blocked with 5% skim milk for 2 h, added with IGF-1 (Abcam, Cambridge, MA, USA) at 1:1000, and blocked at 4°C overnight. After that, the primary antibody was washed away and added with the horseradish peroxidase (HRP)-labeled goat anti-rabbit secondary antibody (Abcam, Cambridge, MA, USA) at 1:5000, incubated at 37°C for 1 h, and rinsed 3 times with phosphate-buffered saline (PBS) for 5 min each. Then, the excess liquid on the membrane was blotted with filter paper, and then developed by enhanced chemiluminescence (ECL). Finally, the protein bands were scanned and the grayscale values were analyzed in Quantity One software, with GAPDH as the internal reference.

Cell Counting Kit-8 (CCK-8) Detection

Cell viability was measured with the help of CCK-8 kit (Beyotime Biotechnology Co., Ltd., Shanghai, China) in strict accordance with the kit instructions. The procedures were as follows: 100 μL cells (2×10^4 cells per well) were seeded on 96-well plates and incubated at 37°C. The optical density was measured at 450 nm by microtitration plate readers at different time points, and the cell viability was expressed as absorbance. The obtained result represented the average of three replicates under the same conditions. In addition, a concentration-dependent curve was generated after culturing in different concentrations of 5-FU for 72 h according to cell viability. Likewise, the obtained result represented the average of three replicates under the same conditions.

Flow Cytometry

The transfected drug-resistant colon cancer cells were cultured with 2 μg/mL 5FU for 48 h before they were collected and digested with 0.25% trypsin. Finishing digestion, the cells were rinsed twice with PBS, added with 100 μL binding buffer, and then configured into 1×10^6/mL suspension. After that, Annexin V-FITC and PI (Yeasen Biotechnology Co., Ltd., Shanghai, China) were added in turn, incubated at room temperature shielded from light for 5 min, and finally FCM FC500MCL was applied for detection. The experiment was repeated for 3 times and averaged.

Statistical Analysis

SPSS 22.0 (IBM, Armonk, NY, USA) was responsible for data processing, and GraphPad 8 was employed to draw the required pictures. The counting data were expressed in the form of (percentage), and the *t*-test was adopted for inter-group comparison. While the measurement data were expressed as (mean±standard deviation), the inter-group comparison was performed by the *t*-test, and the multi-group comparison was conducted by one-way ANOVA and LSD post-hoc test. The diagnostic value was assessed by receiver operating characteristic curve (ROC) curve, and the correlation was analyzed by Pearson correlation coefficient. A statistically significant difference was assumed at $p<0.050$.

Results

Comparison of General Information

Except for a significant difference in dietary preferences ($p<0.001$), no other marked difference was observed in general information between the two groups ($p>0.050$, Table II).

Comparison of IFNG-AS1 and ANRIL Expression Levels

The serum levels of IFNG-AS1 and ANRIL in the RG were markedly higher than those in the CG ($p<0.050$). According to ROC curve analysis, when the cut-off value was 0.725, the diagnostic sensitivity and specificity of IFNG-AS1 for colitis were 57.63% and 84.68% respectively, while the corresponding diagnostic sensitivity and specificity of ANRIL colitis were 57.63% and 90.32% when the cut-off value is 1.685. A binary Logistic regression analysis was carried out with IFNG-AS1 and ANRIL as independent variables, and the combined formula Log (P) =-6.6243.473 × IFNG-AS1+2.882 × ANRIL, was obtained. When the cut-off value was 0.514, the diagnostic sensitivity and specificity of the model for colitis were 72.88% and 83.87%, respectively. Pearson correlation coefficient analysis revealed that IFNG-AS1 and ANRIL were positively correlated in the RG ($r=0.540$, $p<0.001$, Figure 1, Table III)

Pathological Relationship Between IFNG-AS1, ANRIL and Colitis

The analysis revealed that the expression levels of IFNG-AS1 and ANRIL in the RG were not related to age, BMI, gender, or disease types ($p>0.050$), but were closely associated with the disease con-

Table II. Comparison of general information between the two groups.

	RG (n=118)	CG (n=124)	χ^2 or t	p
Age (years old)	48.63±8.16	46.94±9.28	1.502	0.135
BMI (kg/m²)	23.14±2.28	22.98±2.59	0.509	0.611
Gender				
Male	72 (61.02)	68 (54.84)	0.947	0.331
Female	46 (38.98)	56 (45.16)		
History of intestinal diseases			2.424	0.120
Yes	35 (29.66)	26 (20.97)		
No	83 (70.34)	98 (79.03)		
Dietary preference			32.570	<0.001
Spicy	92 (77.97)	52 (41.94)		
Light	26 (22.03)	72 (58.06)		
Exercise habits			1.873	0.171
Yes	16 (13.56)	25 (20.16)		
No	102 (86.44)	99 (79.84)		
Education level			1.168	0.280
<High school	70 (59.32)	65 (52.42)		
≥High school	48 (40.68)	59 (47.58)		

dition and course (p<0.050). Out of 118 patients, there were 10 cases of massive hematostool, 6 cases of intestinal stenosis, 8 cases of intestinal perforation, 2 cases of toxic intestinal dilatation, 5 cases of polyps, 1 case of internal fistula, 2 cases of anal and perianal diseases and 3 cases of other systemic complications during the treatment, with a prevalence of 31.36% (37/118). We then grouped patients with complications as group A and those without complications as group B, the comparison of IFNG-AS1 and ANRIL expression levels in the two groups showed that both IFNG-AS1 and ANRIL expression levels in group A were higher than those in group B (p<0.050). According to ROC curve analysis, when the cut-off value was 0.705, the prediction sensitivity of IFNG-AS1 for complications was 81.08% and the specificity was 53.09%. When the cut-off value was 2.125, the predictive sensitivity and specificity of ANRIL to complications were 43.24% and 88.89% respectively. While IFNG-AS1 combined with ANRIL (Log (P) =-6.485+ -2.787 × IFNG-AS1 + 4.422 × ANRIL) had a predictive sensitivity of 78.38% and specificity of 79.01% for complications when the cut-off value was 0.384. (Tables IV, V, Figure 2)

Effects of IFNG-AS1 on Intestinal Epithelial Cells

After transfection of IFNG-AS1 to intestinal epithelial cells HIEC and HIEC-6, the IFNG-AS1 expression in IFNG-AS1-mimics group was detected to be the highest among the three groups (p<0.050). The CCK-8 experiment showed that the cell proliferation ability of the IFNG-AS1-mimics group was notably reduced, while that of the IFNG-AS1-inhibit group was the strongest (p<0.050). Flow cytometry exhibited that the apoptosis rate in the IFNG-AS1-mimics group was noticeably increased, while that in the IFNG-AS1-inhibit group was reduced (p<0.050). According to WB detection, Bax and Caspase-3 protein elevated and Bcl-2 protein declined in HIEC and HIEC-6 in IFNG-AS1-mimics group (p<0.050), while Bax and Caspase-3 protein decreased and Bcl-2 protein increased in IFNG-AS1-inhibit group (p<0.050, Figure 3)

Effects of ANRIL on Intestinal Epithelial Cells

After transfection of ANRIL to intestinal epithelial cells HIEC and HIEC-6, the ANRIL expression in ANRIL-mimics group was observed to be the highest among the three groups (p<0.050). After detection, the proliferation ability and apoptosis rate of HIEC and HIEC-6 cells were significantly increased by inhibiting ANRIL expression, while Bax and caspase-3 proteins were decreased and bcl-2 protein was increased (p<0.050, Figure 4)

Discussion

Colitis, as an autoimmune disease, currently has a high incidence in the world, and if it is not treated in time, it is likely to develop into colon cancer[14-16].

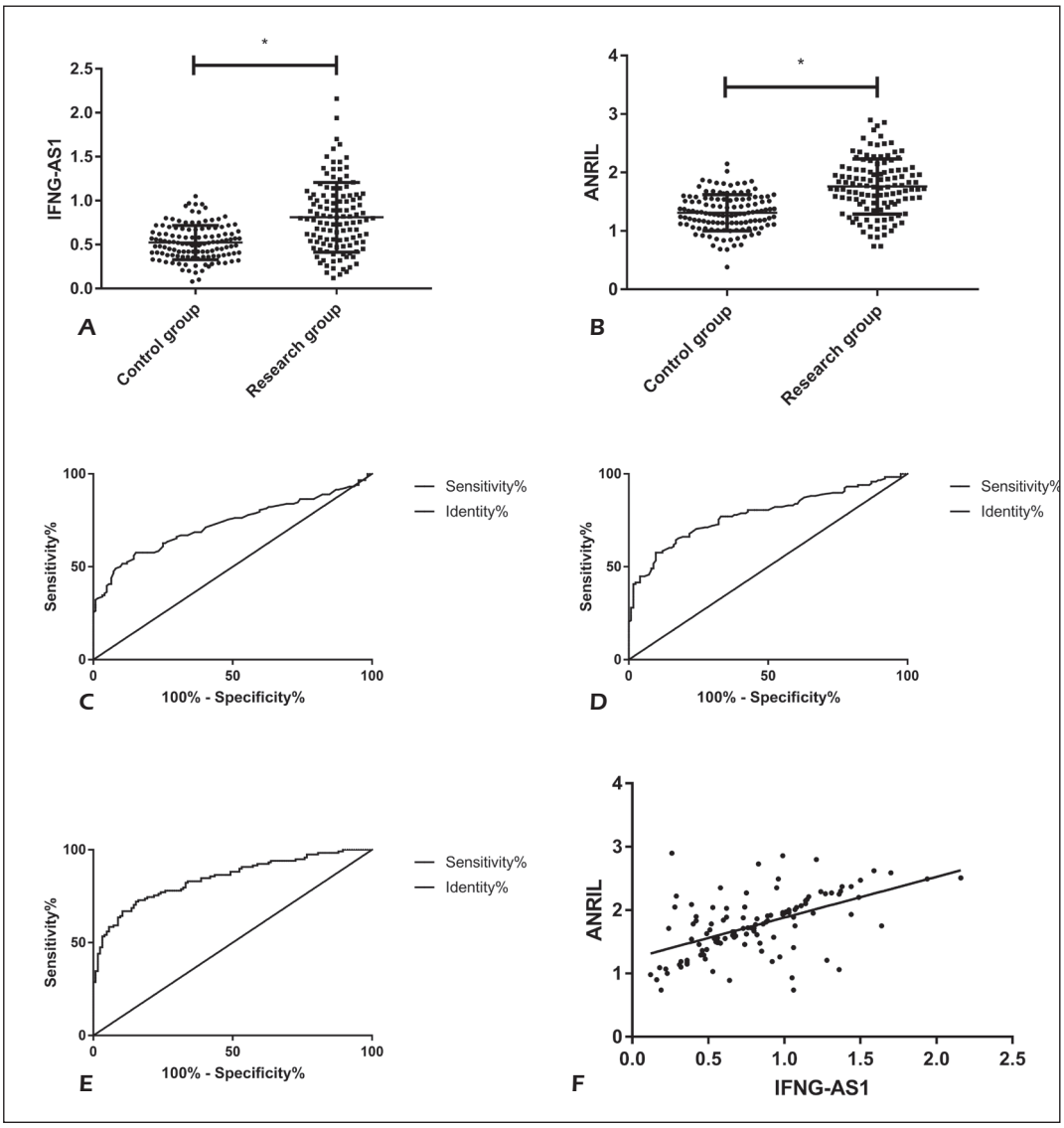

Figure 1. Comparison of IFNG-AS1 and ANRIL expression levels in the two groups. **A**, Comparison of IFNG-AS1 expression level between the two groups. **B**, Comparison of ANRIL expression level between the two groups, *indicated $p<0.050$. **C**, ROC curve of IFNG-AS1 for colitis diagnosis. **D**, ROC curve of ANRIL for colitis diagnosis. **E**, ROC curve of IFNG-AS1 combined with ANRIL for colitis diagnosis. **F**, Correlation analysis between IFNG-AS1 and ANRIL expression levels in the RG.

Table III. Diagnostic value of IFNG-AS1 and ANRIL in colitis.

	IFNG-AS1	ANRIL	IFNG-AS1 combined with ANRIL
AUC	0.729	0.783	0.845
Std. Error	0.033	0.030	0.025
95% CI	0.663-0.794	0.724-0.842	0.795-0.894
Cut-off	>0.725	>1.685	>0.514
Sensitivity (%)	57.63	57.63	72.88
Specificity (%)	84.68	90.32	83.87
Youden index	42.30	47.95	56.75
p	<0.001	<0.001	<0.001

Table IV. Pathological relationship between IFNG-AS1, ANRIL and colitis.

	n	IFNG-AS1	*t* or *F*	*p*	ANRIL	*t* or *F*	*p*
Age (years old)			0.405	0.686		0.375	0.709
<48	56	0.75±0.38			1.82±0.45		
≥48	62	0.78±0.42			1.85±0.42		
BMI (kg/m²)			0.393	0.695		0.356	0.723
<23	52	0.76±0.40			1.83±0.42		
≥23	66	0.79±0.42			1.86±0.48		
Gender			0.449	0.655		0.462	0.645
Male	72	0.76±0.48			1.86±0.43		
Female	46	0.80±0.46			1.82±0.50		
Disease types			0.165	0.869		0.239	0.811
Hemorrhagic	20	0.76±0.40			1.88±0.46		
Ulcerative	98	0.78±0.51			1.85±0.52		
Conditions			5.449	0.006		62.660	<0.001
Mild	29	0.62±0.28			1.48±0.32		
Moderate	58	0.74±0.34			1.64±0.25*		
Severe	31	0.93±0.48*#			2.28±0.37*#		
Course of disease			3.501	0.018		12.200	<0.001
Initial onset	26	0.65±0.22			1.45±0.36		
Chronic relapse	56	0.75±0.28			1.71±0.29@		
Chronic persistence	30	0.76±0.34			1.74±0.32@&		
Acute outbreak	6	1.06±0.21@&^			2.26±0.20@&^		

Note: *represents a comparison with mild conditions, $p<0.050$; #represents a comparison with moderate conditions, $p<0.050$; represents a comparison with the initial onset type, $p<0.050$; &represents a comparison with the chronic recurrence type, $p<0.050$; ^represents a comparison with the chronic recurrence type, $p<0.050$.

At present, the pathogenesis of colitis remains elusive, and understanding its pathogenic factors is of great significance for future clinical diagnosis, treatment and prevention of this disease. Therefore, researchers at home and abroad are constantly devoting themselves to exploring the key factors that affect the occurrence and development of colitis. Wei et al[17] proposed that high-salt diet can stimulate intestinal Th17 response to cause colitis, while Chiaro et al[18] believed that intestinal flora members regulating purine metabolism could aggravate colitis in mice. Though the role of LncRNA in various diseases has been confirmed, the current research on colitis is still scarce. Among them, it is not clear whether IFNG-AS1 and ANRIL, as LncRNA which have been proved to be closely related to inflammatory factors or inflammatory diseases[19,20], have a close relationship with colitis. This study, by exploring the effect of IFNG-AS1 and ANRIL on colitis, is of great clinical significance in future treatment of this disease. One is that in the future, we can judge whether the patients have diseases by detecting IFNG-AS1 and ANRIL, which can effectively assist the clinical detection which is currently lacking serum markers, improve the early diagnosis of colitis, and improve the prognosis of patients. Secondly, by

Table V. Predictive value of IFNG-AS1 and ANRIL for complications.

	IFNG-AS1	ANRIL	IFNG-AS1 combined with ANRIL
AUC	0.723	0.721	0.863
Std. Error	0.050	0.050	0.036
95% CI	0624-0.821	0.624-0.818	0.793-0.933
Cut-off	>0.705	>2.125	>0.384
Sensitivity (%)	81.08	43.24	78.38
Specificity (%)	53.09	88.89	79.01
Youden index	34.17	32.13	57.39
p	<0.001	<0.001	<0.001

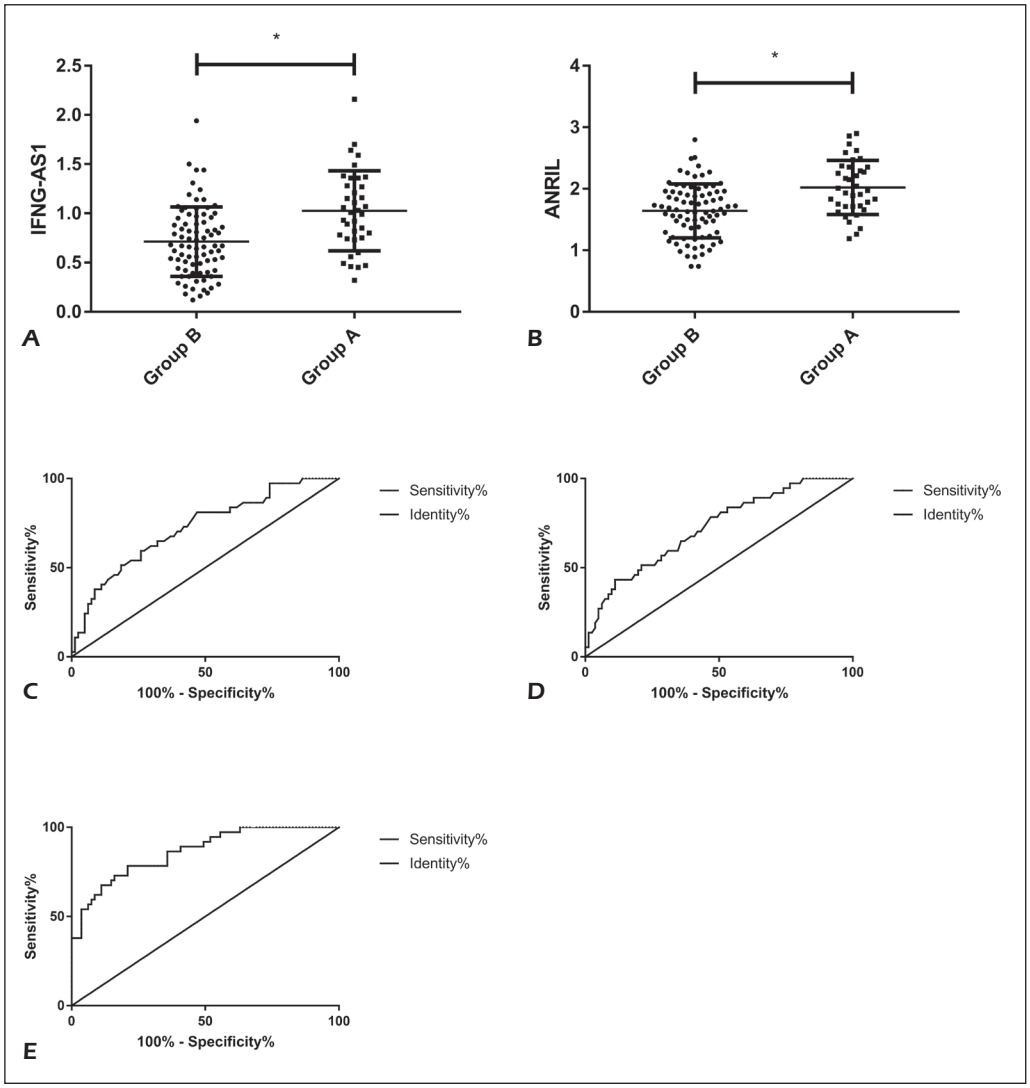

Figure 2. Effects of IFNG-AS1 and ANRIL on complications. **A**, Comparison of IFNG-AS1 expression level between group A and group B. **B**, Comparison of ANRIL expression level between group A and group B. *indicated $p<0.050$. **C**, ROC curve of IFNG-AS1 for predicting complications. **D**, ROC curve of ANRIL for predicting complications. E, ROC curve of IFNG-AS1 combined with ANRIL for predicting complications.

confirming the role of IFNG-AS1 and ANRIL in colitis, we can better understand the disease development process of patients through the above indicators in the future, which provides certain references for clinical judgment of rehabilitation and prognosis of patients, and is also more conducive for doctors to timely carry out corresponding intervention measures. Thirdly, molecular targeted therapy is a hot spot in clinical practice. By exploring the role of IFNG-AS1 and ANRIL in colitis, we hold that in the future, the targeted therapy of IFNG-AS1 and ANRIL may achieve better results than the current clinical treatment.

The results of this investigation showed that IFNG-AS1 and ANRIL were highly expressed in peripheral blood of patients with colitis, suggesting that the two may be involved in the occurrence or development of colitis, which was consistent with Rankin et al[21] and Zhang et al[22] in exploring the effects of IFNG-AS1 and ANRIL on lung cancer and inflammatory bowel disease, and can support the results of this study. In addition, the ROC curve analysis indicated that the combined detection of IFNG-AS1 and ANRIL had a predictive sensitivity of 72.88% and a specificity of 83.87% for the occurrence of colitis, suggesting their roles as clinical screening indicators for coli-

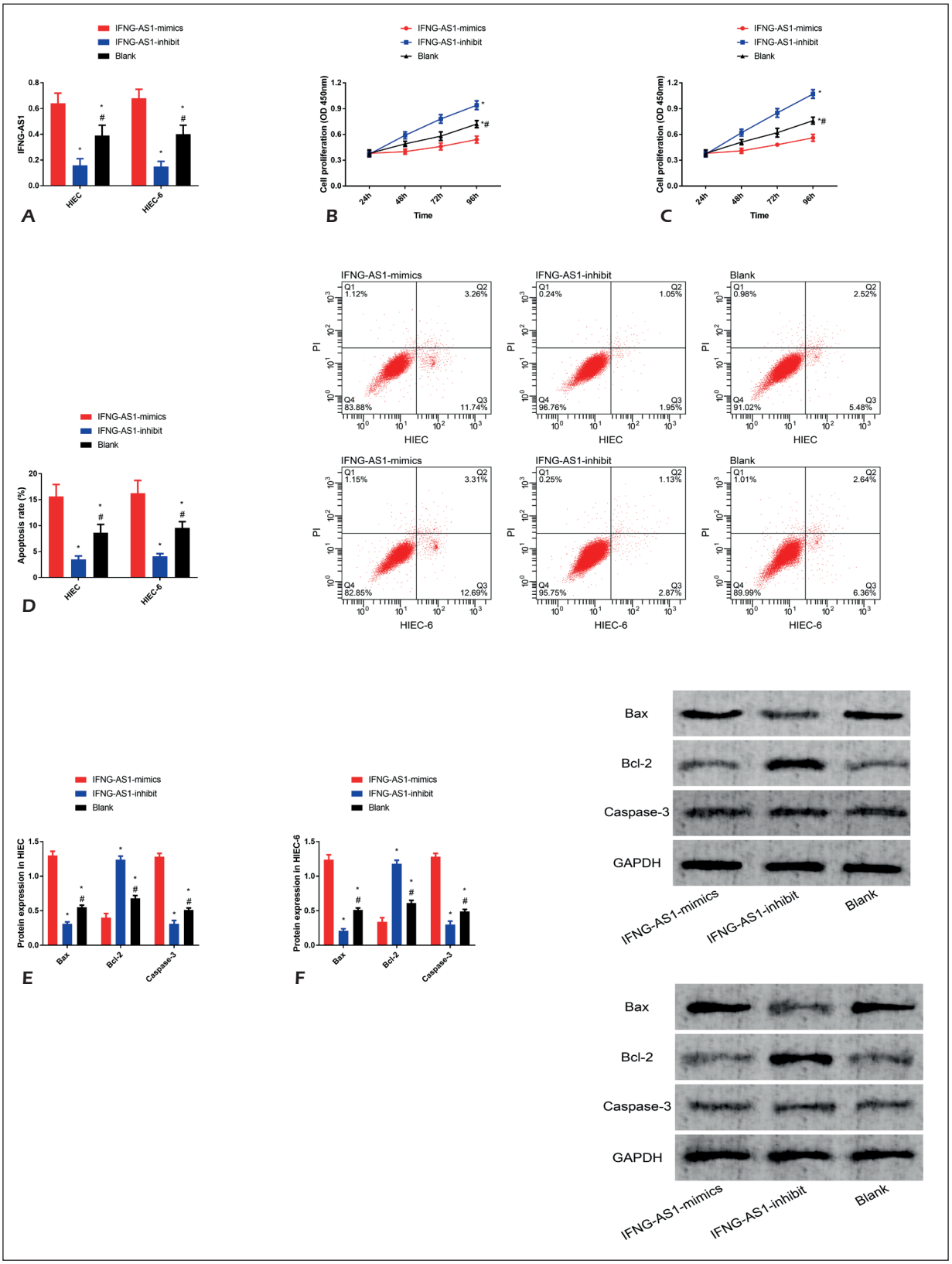

Figure 3. Effects of IFNG-AS1 on intestinal epithelial cells. **A**, IFNG-AS1 expression level in HIEC and HIEC-6 after transfection of IFNG-AS1. **B**, The proliferation of HIEC cells. **C**, The proliferation of HIEC-6 cells. **D**, Apoptosis rate and flow cytometry of HIEC and HIEC-6 cells. **E**, Protein expression in HIEC and Western Blot. **F**, Protein expression in HIEC-6 and Western Blot.

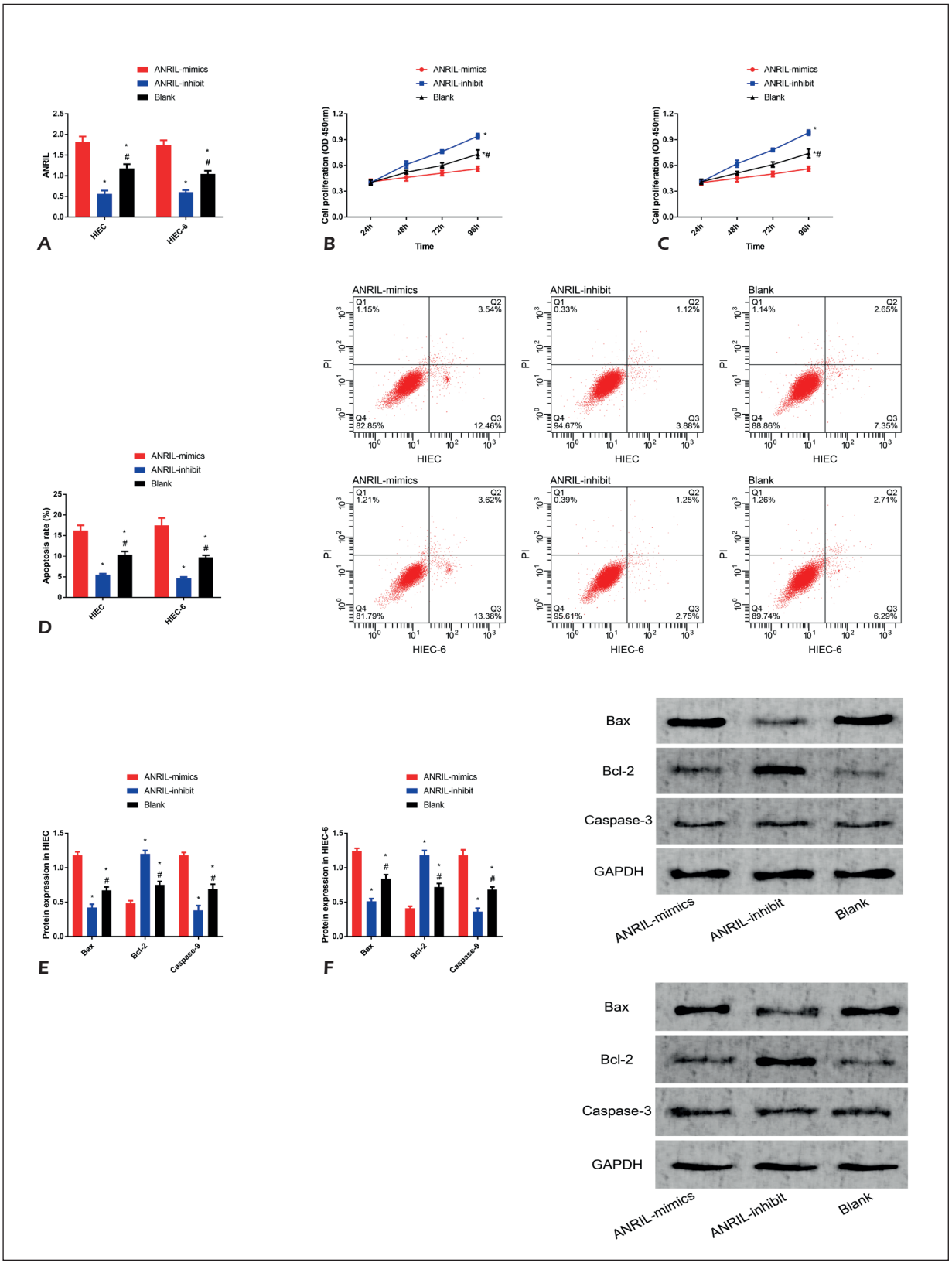

Figure 4. Effects of ANRIL on intestinal epithelial cells. **A**, The expression level of ANRIL in HIEC and HIEC-6 after transfection with ANRIL. **B**, The proliferation of HIEC cells. **C**, The proliferation of HIEC-6 cells. **D**, Apoptosis rate and flow cytometry of HIEC and HIEC-6 cells. **E**, Protein expression in HIEC and Western Blot. F, Protein expression in HIEC-6 and Western Blot.

tis in the future to improve the early detection rate of colitis and enhance the prognosis of patients. IFNG-AS1 can be used as a diagnostic indicator of cardiovascular diseases[23], which further confirms the value of IFNG-AS1 and ANRIL as blood markers in future clinical applications. Today, common blood markers in clinical practice are characterized by high sensitivity but insufficient specificity (such as CRP, BNP). While the case in colitis is that it is not only an autoimmune disease, but also an inflammatory disease. Early clinical detection of colitis is an intricate process that involves many indicators and complex detection methods, and pathological biopsy is needed for final diagnosis[24], which may explain why most patients miss the best treatment time at the initial stage of the disease. The combined detection of IFNG-AS1 and ANRIL in our study reveals that, the two not only have a high sensitivity, but also enjoy a specificity of 83.87% for the identification of colitis, which provide a great help in early clinical screening. What's more, we found a positive correlation between IFNG-AS1 and ANRIL through correlation analysis, which also showed that the two have a consistent change trend in colitis, and it was speculated that their effects on colitis may be similar. Furthermore, we observed the relationship between the two and the clinicopathological features of colitis, and noticed that they were closely related to the patient's condition and course of disease, validating our initial hypothesis that IFNG-AS1 and ANRIL were involved in the pathogenesis of colitis. Moreover, analysis of the occurrence of complications during the treatment process also demonstrated that there was a certain relationship with IFNG-AS1 and ANRIL, and the combined detection of the two had a good predictive value for complications in patients with colitis.

IFNG-AS1 and ANRIL have been proved to be implicated in the occurrence and development of many diseases. For the sake of further understanding the mechanism of IFNG-AS1 and ANRIL in colitis, we transfected the two into intestinal epithelial cells to detect the biological behavior of cells. The results exhibited that inhibited IFNG-AS1 and ANRIL expression remarkably increased cell proliferation and decreased apoptosis rate, suggesting that the main pathogenic mechanism of IFNG-AS1 and ANRIL in colitis was by promoting cell apoptosis and inhibiting cell proliferation. This agrees with the research of Liu et al[25] and Thomas et al[26], which can support our experimental results. Apart from that, IFNG-AS1 and ANRIL can serve as clinical targets for future colitis for clinical treatment. Zhang et al[27] pointed out that ANRIL can be used as a therapeutic target for pediatric medulloblastoma, which is similar to our point of view. Wang et al[28] indicated that ANRIL inhibitors have certain therapeutic effects on nasopharyngeal carcinoma, which further verified the future clinical value of IFNG-AS1 and ANRIL as therapeutic targets. However, in the process of application, further experiments are needed to determine the dose of inhibitors.

At present, the best treatment plan for patients with more severe colitis (such as massive bleeding, toxic colitis, or continuous exacerbation) is surgical treatment, which can achieve radical cure of colitis through total colorectal and rectal resection or permanent terminal ileostomy. However, invasive surgical procedures inevitably result in adverse reactions such as infection and oxidative stress injury, and the surgical method has a greater impact on the patient's prognostic quality of life. Therefore, the search for effective conservative treatment plan is also of great significance for future severe colitis.

The purpose of this study was to investigate the expression and effect of IFNG-AS1 and ANRIL on the intestinal epithelial cells of colitis patients, but due to limited experimental conditions, there are still inadequacies. To begin with, we are unable to determine how IFNG-AS1 and ANRIL affect colitis. Moreover, no more detailed grouping of colitis types was conducted in this study to determine the clinical value of IFNG-AS1 and ANRIL in different types of colitis. Last but not the least, owing to the short experimental period, we cannot judge the effect of IFNG-AS1 and ANRIL on long-term prognosis of patients. We will conduct a deeper and comprehensive analysis of the above shortcomings as soon as possible to obtain more complete experimental results, so as to contribute to the future clinical diagnosis and treatment of colitis.

Conclusions

To sum up, IFNG-AS1 and ANRIL are highly expressed in colitis, and inhibiting their expression can promote the proliferation of intestinal epithelial cells and reduce apoptosis, which may be potential therapeutic targets for Crohn's colitis in the future.

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Conflict of Interests

The authors declare that they have no conflict of interests.

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