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LncRNA *GAS5* regulates the inflammatory response in inflammatory bowel disease *via* targeting the miR-23a-3p/*BVES* axis

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Abstract

Introduction: *Inflammatory bowel disease (IBD) is an inflammatory pathological condition for which effective drugs are currently lacking. The objective of this study was to reveal the regulatory mechanisms of growth arrest specific transcript 5 (GAS5) in IBD.*

Material and methods: *To mimic IBD in vitro, human fetal colon (FHC) cells were exposed to lipopolysaccharide (LPS) to induce inflammation. The MTT method and flow cytometry were utilized to detect cell viability and apoptosis, respectively. Enzyme-linked immunosorbent assay was performed to determine cytokine concentrations. A luciferase reporter kit was utilized to confirm the binding between GAS5 and the microRNA miR-23a-3p, and between vascular epicardial substance (BVES) and miR-23a-3p.*

Results: *GAS5 and BVES were lowly expressed in the colonic mucosal tissues obtained from patients with IBD, while miR-23a-3p was abundantly expressed. Both GAS5 upregulation and miR-23a-3p inhibition promoted proliferation, impeded apoptosis and abolished inflammatory cytokine release in FHC cells. The expression levels of miR-23a-3p and GAS5 and those of BVES and miR-23a-3p in the colonic mucosa of IBD patients were negatively correlated. GAS5 decreased the level of miR-23a-3p via direct targeting. BVES was targeted and suppressed by miR-23a-3p. Lastly, GAS5 promoted FHC cell proliferation, impeded apoptosis and inhibited cytokine release by upregulating BVES.*

Conclusions: *GAS5 promoted cell viability, impeded apoptosis, and inhibited inflammation in colonic mucosal cells exposed to LPS by targeting miR-23a-3p and then promoting BVES expression. These findings imply that GAS5 could be further explored as a target for IBD.*

Key words: *inflammatory bowel disease, growth arrest-specific transcript 5, blood vessel epicardial substance, inflammation.*

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Introduction

Inflammatory bowel disease (IBD), a chronic, remitting inflammatory disorder in the gastrointestinal tract, consists of two kinds: Crohn's disease and ulcerative colitis. Crohn's disease affects the gastrointestinal tract in a non-continuous manner and causes transmural inflammation, while ulcerative colitis is typified by inflammation in the mucosa and limited to the colon [1, 2]. Although the etiology of IBD remains elusive, it appears to persist in genetically susceptible individuals due to an imbalanced immune response against intestinal microorganisms [3]. Recent evidence suggests that the pathogenesis of IBD results from the interplay of genetic susceptibility and environmental impacts on the microbiome, which, through a weakened intestinal barrier, lead to inappropriate immune activation in the intestines [4]. Inflammatory bowel disease results in a heavy cost to the health-care system [4]. Currently, some drugs targeting interleukin (IL)-23, tumor necrosis factor α (TNF-α), and IL-12 are used in IBD treatment. However, many patients do not respond to these drugs, which have various side effects [5, 6]. Hence, it is important to explore the pathogenesis of IBD and find an effective target for disease treatment.

Long non-coding RNAs (lncRNAs), defined as non-coding transcripts with a length of over 200 nucleotides, are indispensable regulators in the progression of many diseases,

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such as virus infection, malignant tumors, and IBD. They exert a key regulatory effect on gene expression [7-9]. In accordance with previous genome-wide transcriptome profiling, a great number of lncRNAs are differentially expressed between inflamed and non-inflamed colon pinch biopsies [10]. Some lncRNAs, such as colon cancer-associated transcript-1, have been shown to regulate the development of IBD *via* binding and inhibiting microRNA (miRNA) expression [9]. Growth arrest specific transcript 5 (*GAS5*) is an lncRNA that was downregulated in the inflamed biopsies of IBD patients compared to non-inflamed biopsies [11]. Moreover, a significant increase in the level of *GAS5* has been identified as the rate-limiting factor in the remission of at least 20% of IBD patients who exhibit resistance to GC treatment [12]. In addition, *GAS5* has been reported to regulate intestinal matrix metalloproteinases in pediatric patients with IBD [13]. Whether *GAS5* could regulate other key steps in IBD development remains unclear. The microRNA miR-23a-3p is overexpressed in plasma samples of patients diagnosed with ulcerative colitis, and its expression is positively correlated with disease severity [14]. The regulatory effect of miR-23a-3p has also been observed in intestinal ischemia/reperfusion rat models [15]. Nevertheless, the functional roles of miR-23a-3p in IBD remain unclear. Blood vessel epicardial substance (*BVES*) belongs to the Popeye-domain-containing family of tight-junction associated proteins [16]. A recent study demonstrated that the *BVES* mRNA level was downregulated in human ulcerative colitis biopsy samples, and it may play a protective role in ulcerative colitis and infectious colitis [17]. Furthermore, it is considered a prerequisite for the maintenance of colonic epithelial integrity in experimental colitis by modifying intestinal permeability [17]. However, the involvement of *BVES* in IBD development has not been explored.

In this study, we analyzed the levels of *GAS5*, miR-23a-3p, and *BVES* in colonic mucosal samples of IBD, aiming to reveal the regulatory mechanism of *GAS5* in the proliferation, apoptosis, and pro-inflammatory cytokine secretion in an IBD cell model. Our results identified *GAS5* as a critical inhibitor of IBD.

Material and methods

Participants

Ten patients with IBD and 10 healthy volunteers were recruited from our hospital in the period from August 2023 to April 2024. The inclusion and exclusion criteria for IBD patients were as follows [18]: 1) aged 18 years or older; 2) the diagnosis of IBD was in accordance with their clinical characteristics, histological results, radiological findings, and endoscopic examination by an experienced doctor. All participants who met the following exclusion criteria were excluded: 1) chronic diarrhea due to other causes; 2) malignant tumors; 3) concurrent autoimmune or endocrine diseases; 4) history of persistent infections; 5) pregnant or breastfeeding women; 6) patients using estrogen for any reason. The IBD group consisted of 6 males and 4 females, with an average age of 34.06 ±3.88 years and a body mass index (BMI) of 22.50 ±1.67. The control group included 5 males and 5 females, with an average age of 45.22 ±8.42 years and a BMI of 24.56 ±4.62. Colonic mucosal tissues were obtained from healthy volunteers and IBD patients during the endoscopy. The study protocol received approval by the Ethics Committee of the First Affiliated Hospital, Anhui University of Science and Technology (approval number: 2023-KY-150-001) and adhere to principles outlined in the Declaration of Helsinki. Written informed consent was collected from all participants.

Cell line

The human fetal colon (FHC) cell line was purchased from ATCC (USA). RPMI-1640 medium (Solarbio, Beijing, China) was used to culture the FHC cells. The FHC cell line was grown in a 5% CO_2 incubator at 37°C. For cell culture, RPMI-1640 medium was mixed with 1% streptomycin/penicillin and 10% fetal bovine serum (both from Thermo Fisher Scientific, USA). To induce *in vitro* inflammation, lipopolysaccharide (LPS; Sigma, USA) at a dose of 1 μg/ml was added to FHC cells and cultured for 24 h [19]. Lipopolysaccharide treatment was applied to all trials, and it was done after transfection. Cells were washed with phosphate-buffered saline three times, each for 10 min, before each use.

Cell transfection

The vectors overexpressing *GAS5*, vectors overexpressing *BVES*, empty vector, miR-23a-3p inhibitor, negative control (NC) inhibitor, mimic NC, miR-23a-3p mimic, and vectors expressing short hairpin RNA targeting *BVES* (sh-*BVES*) or control short hairpin RNA (sh-NC) (all from GenePharma, China) were delivered into FHC cells utilizing Lipofectamine 3000 (Invitrogen, USA). Transfected cells were incubated 48 h before use in each assay.

MTT assay

The human fetal colon cell viability was quantified using the MTT method. Transfected FHC cells (6×10^3 cells/well) were planted into 96-well plates and subjected to LPS treatment as described above. After 6-72 h, cells were maintained with 10 μl of MTT solution (Solarbio) for 4 hours. Subsequently, 150 μl of DMSO was added and incubated with cells for 10 min to dissolve the violet crystals. Subsequently, the optical density values of cells at 490 nm were detected, and the cell vitality at different time points was analyzed.

Flow cytometric analysis

An Annexin V-FITC/PI apoptosis detection kit (Beyotime, China) was purchased to determine the apoptosis rate of FHC cells. A total of 3×10^5 transfected FHC cells were incubated with Annexin V-FITC and PI (each 5 μl) for 5 min in a light-proof room. Then, the cell apoptotic

rate was determined utilizing FlowJo (TreeStar, USA) on a flow cytometer.

Western blotting assay

Protein samples (20 μg each) was separated on 12% SDS-PAGE gels, transferred onto PVDF membranes (Millipore Sigma, USA), and incubated with non-fat milk (5%) for 50 min in an ambient environment. Subsequently, these membranes were maintained at 4°C with anti-BVES (PA5-80386, 1 : 1000, CST, USA) and anti-GAPDH (ab181602, 1 : 1000, Abcam, UK) overnight. Subsequently, the membranes were maintained with a secondary antibody (goat anti-rabbit, ab181662, 1 : 2000, Abcam) at 37°C for 50 min. Finally, an ECL kit (Takara, Japan) was used to visualize the protein bands, and the blots were analyzed using Image J. The expression level of GAPDH was used to normalize that of BVES.

Real-time quantitative PCR (RT-qPCR)

Total RNA was isolated from colonic mucosal tissue specimens and FHC cells by TRIzol reagent (Invitrogen). Subsequently, the cDNA was reverse transcribed from RNA using a cDNA Reverse Transcription Kit (Invitrogen) in accordance with the following conditions: 95°C, 10 min; 42°C, 2 min; 37°C, 15 min; 85°C, 5 s; and then 4°C, 30 min. Subsequently, a SYBR PrimeScript RT-qPCR kit (Takara) was used for RT-qPCR, which was performed on an ABI 7500 system (ABI, USA). The primers in this experiment were as follows: *GAS5*, 5′-GTGAGGTATGGTGCTGGGTG-3′ (F) and 5′-GCCAATGGCTTGAGTTAGGC-3′ (R); miR-23a-3p, 5′-GCCGAGATCACATTGCCAGGG-3′ (F) and 5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC GGAAAT-3′ (R); *BVES* 5′-CCTCTGCACACAGATCTCCA-3′ (F) and 5′-CAAGGCAGCTGATGGACTTT-3′ (R). The expression of *GAS5* and *BVES* was normalized to *GAPDH* (5′-CCAGGTGGTCTCCTCTGA-3′ [F] and 5′-GCTGTAGCCAAATCGTTGT-3′ [R]). The expression of miR-23a-3p was normalized to *U6* (5′-CTCGCTTCGGCAGCACA-3′ [F] and 5′- AACGCTTCACGAATTTGCGT-3′ [R]). The gene expression levels were quantified by the $2^{-\Delta\Delta Ct}$ method.

Luciferase reporter analysis

The putative binding of miR-23a-3p to *GAS5* or the 3′ prime untranslated region (3′-UTR) of *BVES* was verified by luciferase reporter analysis. The mutant (mut) or wide type (wt) sequences of *GAS5* or the 3′-UTR of *BVES* that harbor the binding locations of miR-23a-3p were delivered into the vector pmirGLO to construct the GAS5-wt reporter plasmid, GAS5-mut reporter plasmid, BVES-wt reporter plasmid, and BVES-mut reporter plasmid. Then, these reporter plasmids were transfected with inhibitor NC, miR-23a-3p inhibitor, mimic NC, or miR-23a-3p mimic into FHC cells. At 48 h after cell transfection, the activities of luciferase were examined.

Enzyme-linked immunosorbent assays (ELISA)

The content of proinflammatory cytokines in FHC cell supernatant (1×10^6 per sample) were measured by the human IL-1β, TNF-α, and IL-6 ELISA kits (Beyotime, China). All procedures were in line with the manuals and repeated at least three times.

Statistical analysis

Each experiment was conducted at least three times. GraphPad Prism 5 (USA) was utilized for data analysis. All results were presented as mean ± standard deviation. Student's *t*-test was employed for comparing significant differences between two independent groups, while ANOVA (one-way) was applied for multi-group analysis. Pearson's correlation coefficient determined the relationships between the expression levels of *GAS5*, miR-23a-3p, and *BVES* in IBD tissue specimens. *P*-values lower than 0.05 indicated statistical significance: $***p < 0.001$, $**p < 0.01$, $*p < 0.05$.

Results

Overexpression of *GAS5* promoted FHC cell proliferation, impeded apoptosis and inhibited inflammatory cytokine release

Firstly, *GAS5* showed lower expression in the lesion tissues of IBD than in normal tissues (Fig. 1A). Moreover, the *GAS5* expression in FHC cells was suppressed by LPS stimulation compared with the control (Fig. 1B). Next, we transfected the *GAS5* overexpression plasmid and empty vector into LPS-treated FHC cells. The *GAS5* expression was markedly upregulated after *GAS5* overexpression plasmid transfection (Fig. 1C). The MTT assay, flow cytometry, and ELISA showed that *GAS5* overexpression significantly increased FHC cell viability (Fig. 1D), impeded apoptosis (Fig. 1E), and hindered the release of TNF-α and IL-1β, as well as IL-6 (Fig. 1F). Collectively, these data demonstrate that *GAS5* overexpression promotes proliferation and inhibits apoptosis and inflammatory cytokine release in LPS-treated FHC cells.

MiR-23a-3p inhibition promoted FHC cell proliferation, impeded apoptosis and inhibited inflammatory cytokine release

MiR-23a-3p was more abundantly expressed in colonic mucosa of IBD patients than in normal colonic mucosal tissues (Fig. 2A). To explore the functional role of miR-23a-3p in FHC cell viability and apoptosis, we transfected miR-23a-3p inhibitor and inhibitor NC into LPS-treated FHC cells. MiR-23a-3p was robustly downregulated by

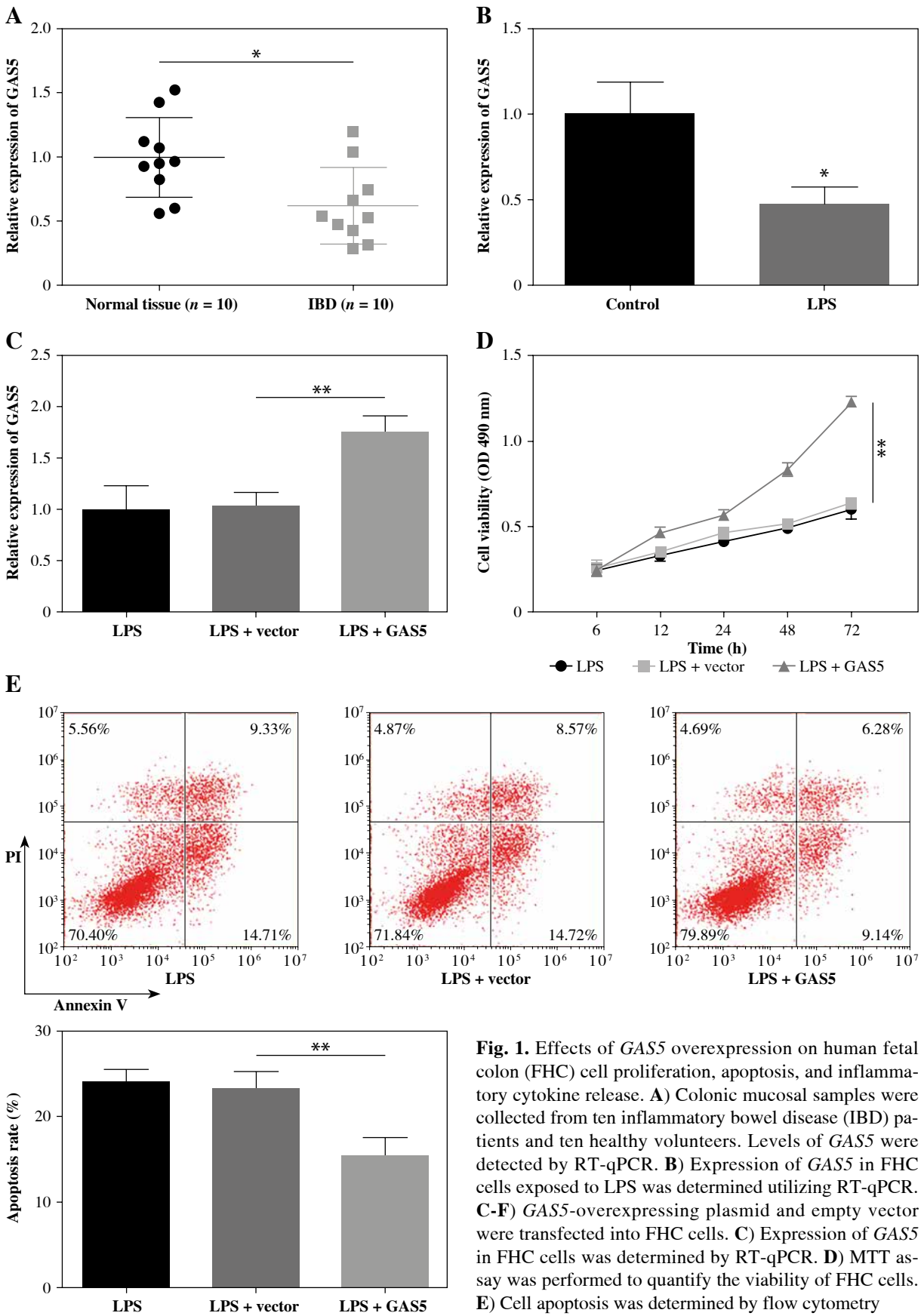

Fig. 1. Effects of *GAS5* overexpression on human fetal colon (FHC) cell proliferation, apoptosis, and inflammatory cytokine release. **A**) Colonic mucosal samples were collected from ten inflammatory bowel disease (IBD) patients and ten healthy volunteers. Levels of *GAS5* were detected by RT-qPCR. **B**) Expression of *GAS5* in FHC cells exposed to LPS was determined utilizing RT-qPCR. **C-F**) *GAS5*-overexpressing plasmid and empty vector were transfected into FHC cells. **C**) Expression of *GAS5* in FHC cells was determined by RT-qPCR. **D**) MTT assay was performed to quantify the viability of FHC cells. **E**) Cell apoptosis was determined by flow cytometry

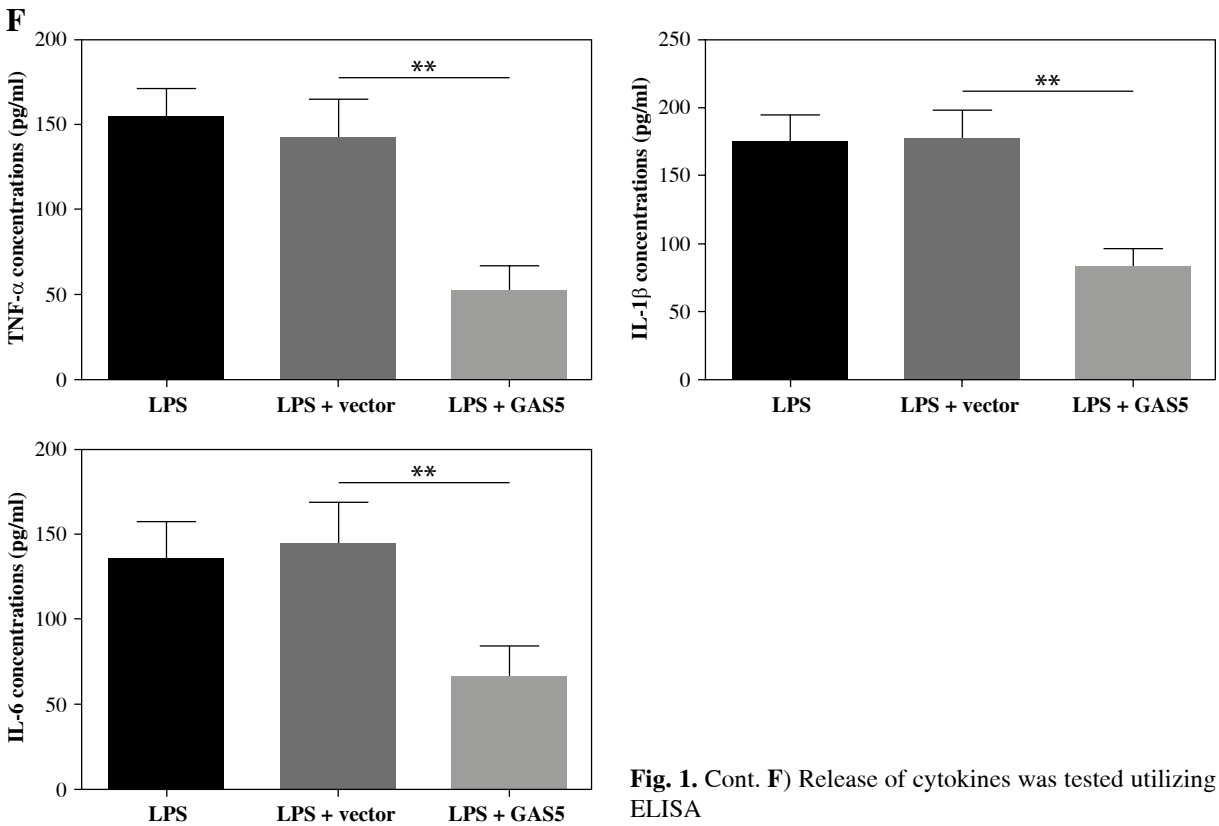

Fig. 1. Cont. **F**) Release of cytokines was tested utilizing ELISA

the delivery of inhibitor targeting miR-23a-3p (Fig. 2B). Our findings proved that miR-23a-3p inhibition markedly enhanced FHC cell viability (Fig. 2C), impeded apoptosis (Fig. 2D), and reduced the release of all three inflammatory cytokines (Fig. 2E). These observations suggest that miR-23a-3p inhibition enhances proliferation and inhibits apoptosis and inflammatory cytokine release in LPS-treated FHC cells.

GAS5 targets miR-23a-3p and suppresses its expression

The expression levels of *GAS5* and miR-23a-3p in the colonic mucosa of IBD patients were negatively correlated (Fig. 3A). The StarBase database predicted a putative binding location between *GAS5* and miR-23a-3p (Fig. 3B). Further analysis revealed that miR-23a-3p mimic effectively reduced the activity of *GAS5*-wt instead of *GAS5*-mut. Concomitantly, inhibition of miR-23a-3p notably upregulated the activity of *GAS5*-wt instead of *GAS5*-mut (Fig. 3C). In addition, *GAS5* overexpression markedly suppressed the miR-23a-3p expression in FHC cells exposed to LPS (Fig. 3D). In summary, *GAS5* suppresses miR-23a-3p expression *via* direct targeting.

MiR-23a-3p targets *BVES* and hinders its expression

BVES showed lower expression in IBD colonic mucosa than in healthy tissues (Fig. 4A). The mRNA levels of *BVES* and miR-23a-3p were negatively correlated in colonic mucosa of IBD patients (Fig. 4B). The TargetScan database predicted a putative binding location between the 3′-UTR of *BVES* and miR-23a-3p (Fig. 4C). The binding was then proven by the luciferase activity method. The luciferase activity of the *BVES*-wt group, not the *BVES*-mut group, was downregulated by increasing miR-23a-3p and upregulated through decreasing miR-23a-3p (Fig. 4D). We further detected the expression of *BVES* in LPS-exposed FHC cells delivered with inhibitor NC or miR-23a-3p inhibitor. As shown in Figure 4E, F, silencing of miR-23a-3p led to notable upregulation of *BVES* expression at both gene and protein levels. Overall, *BVES* is targeted and inhibited by miR-23a-3p.

GAS5 promotes proliferation and inhibits apoptosis and inflammatory cytokine release by upregulating *BVES*

Lastly, we used vectors expressing *BVES* shRNA to transfect LPS-treated FHC cells delivered with the

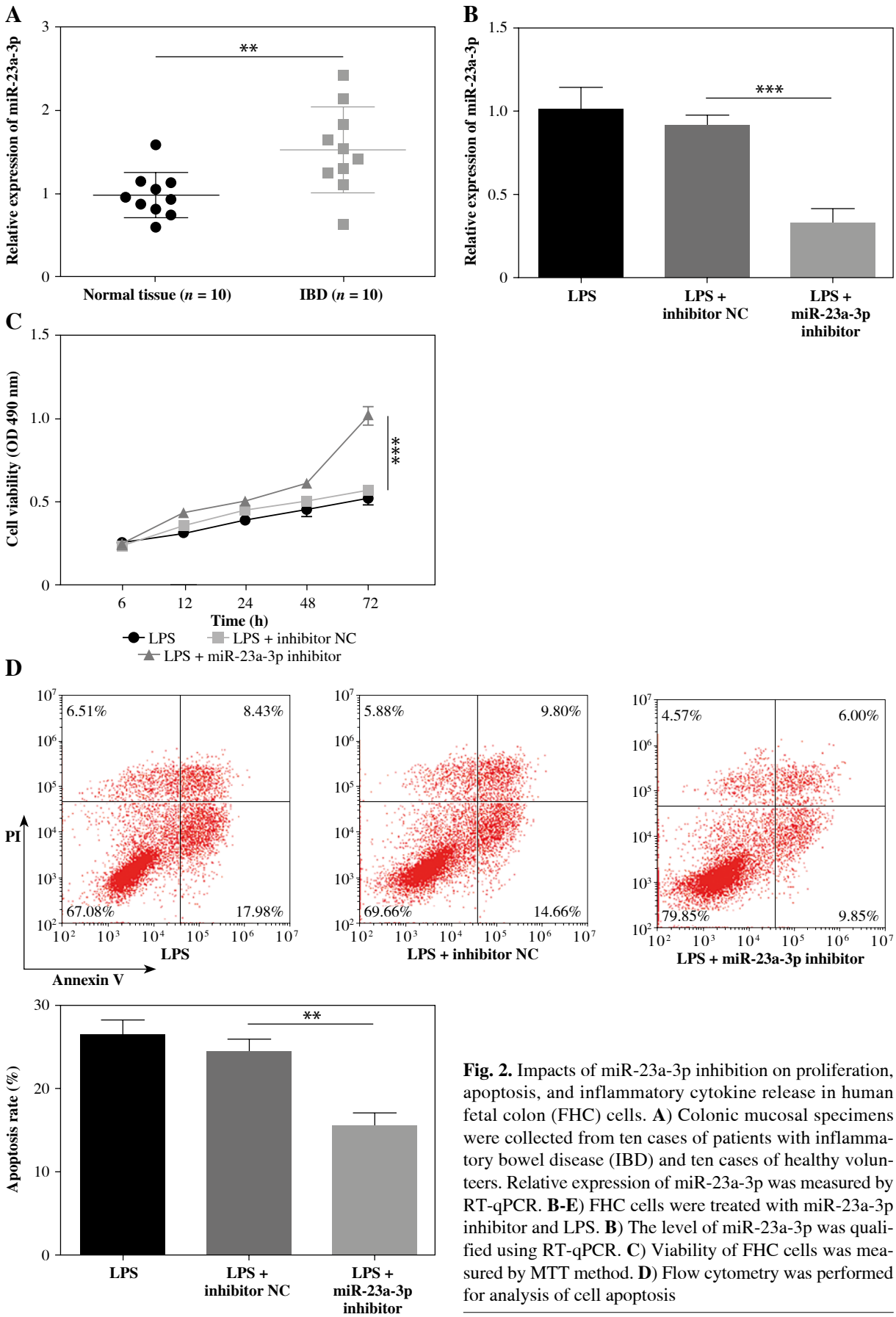

Fig. 2. Impacts of miR-23a-3p inhibition on proliferation, apoptosis, and inflammatory cytokine release in human fetal colon (FHC) cells. **A**) Colonic mucosal specimens were collected from ten cases of patients with inflammatory bowel disease (IBD) and ten cases of healthy volunteers. Relative expression of miR-23a-3p was measured by RT-qPCR. **B-E**) FHC cells were treated with miR-23a-3p inhibitor and LPS. **B**) The level of miR-23a-3p was qualified using RT-qPCR. **C**) Viability of FHC cells was measured by MTT method. **D**) Flow cytometry was performed for analysis of cell apoptosis

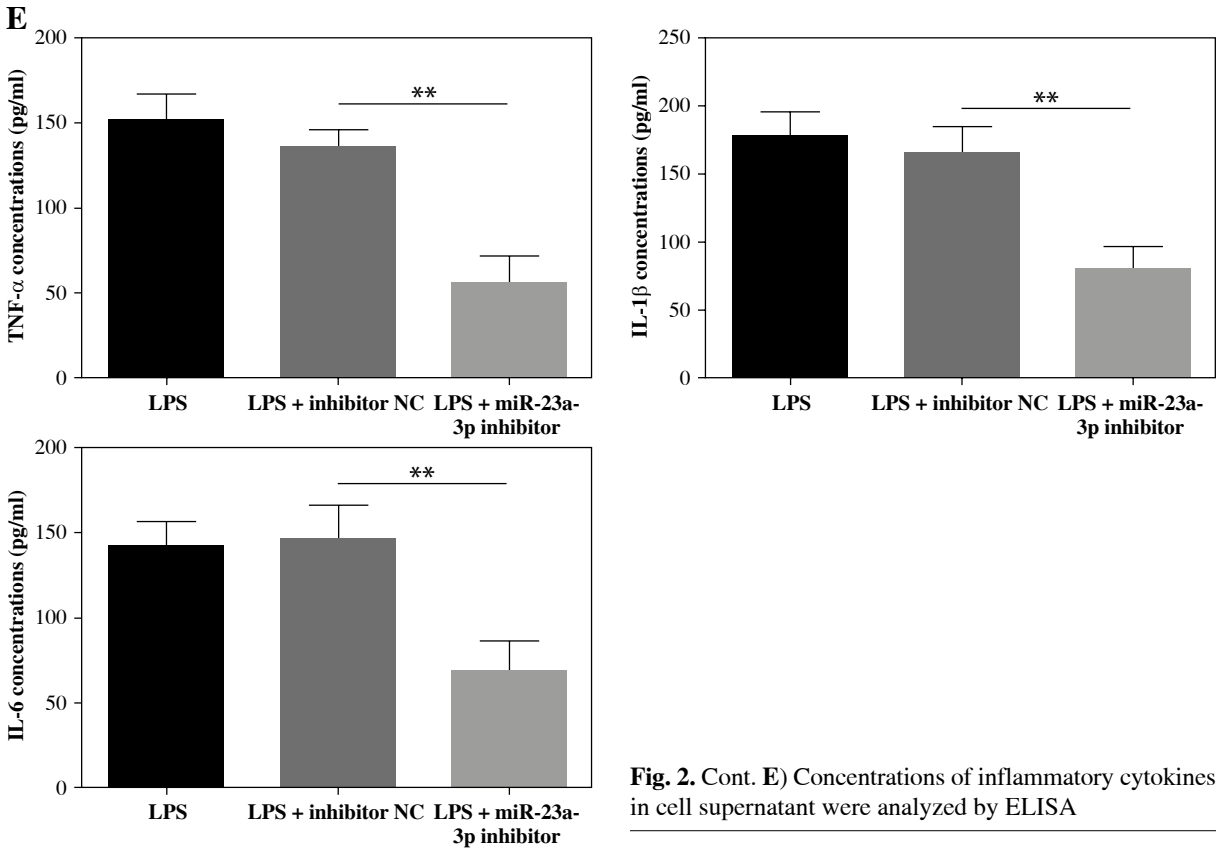

Fig. 2. Cont. **E**) Concentrations of inflammatory cytokines in cell supernatant were analyzed by ELISA

GAS5-overexpressing plasmid or empty vector. The expression of *GAS5* was notably promoted, whereas miR-23a-3p was hindered by the transfection of *GAS5*-overexpressed plasmid (Fig. 5A, B). Meanwhile, both BVES protein and *BVES* mRNA were upregulated after *GAS5* overexpression plasmid transfection, which were partly reversed after sh-*BVES* transfection (Fig. 5C, D). Importantly, our data suggested that *GAS5*'s impacts on FHC cell viability and apoptosis were partly rescued by *BVES* silencing (Fig. 5E, F). The inhibition of cytokine production by *GAS5* overexpression in LPS-treated FHC cells was also reversed by *BVES* silencing (Fig. 5G). Overall, *GAS5* promoted FHC cell viability, and inhibited cell apoptosis and inflammation through upregulating *BVES* expression.

Discussion

Increasing evidence has suggested that lncRNA is a potential target for the therapy of many disorders. Here, we observed downregulated expression of *GAS5* and *BVES* and upregulation of miR-23a-3p in IBD colonic mucosal tissue specimens. Moreover, increased *GAS5* expression facilitated the proliferation, suppressed the apoptosis, and reduced pro-inflammatory cytokine production of FHC cells exposed to LPS *via* inhibiting miR-23a-3p and promoting *BVES* expression.

LncRNAs do not encode peptides or proteins but have two- and three-dimensional structure, which is different from other non-coding RNAs, such as miRNAs, circular RNAs, and small interfering RNAs [20]. LncRNAs are crucial regulators of gene expression involved in multiple biological process, such as cell proliferation, cell cycle, invasion, and drug resistance [21]. LncRNAs act as endogenous inhibitors, also known as spongers, of miRNA in cells to prevent the target miRNA from binding to its downstream targets, thereby promoting expression of this targets [22]. Nie *et al.* reported that lncRNA ITSN1-2 was increased in IBD intestinal mucosal tissues, and participated in activation and differentiation of T cells through facilitating IL-23R expression *via* acting as a miR-125a sponge [23]. Yang *et al.* reported that lncRNA *CRNDE* promoted dextran sulfate sodium-induced apoptosis of a colonic epithelial cell line *via* sponging miR-495 and increasing the level of the suppressor of cytokine signaling 1 [24]. *GAS5* has been reported to exert an anti-inflammatory effect in sepsis [25]. In addition, administration of *GAS5* decreased neuroinflammation in aged mice [26]. Moreover, *GAS5* suppressed inflammatory responses and apoptosis of alveolar epithelial cells in mice with LPS-induced acute lung injury [27]. The present study showed that *GAS5* was decreased in the colonic mucosal samples of IBD patients. Increased expression of *GAS5* improved FHC cell viability,

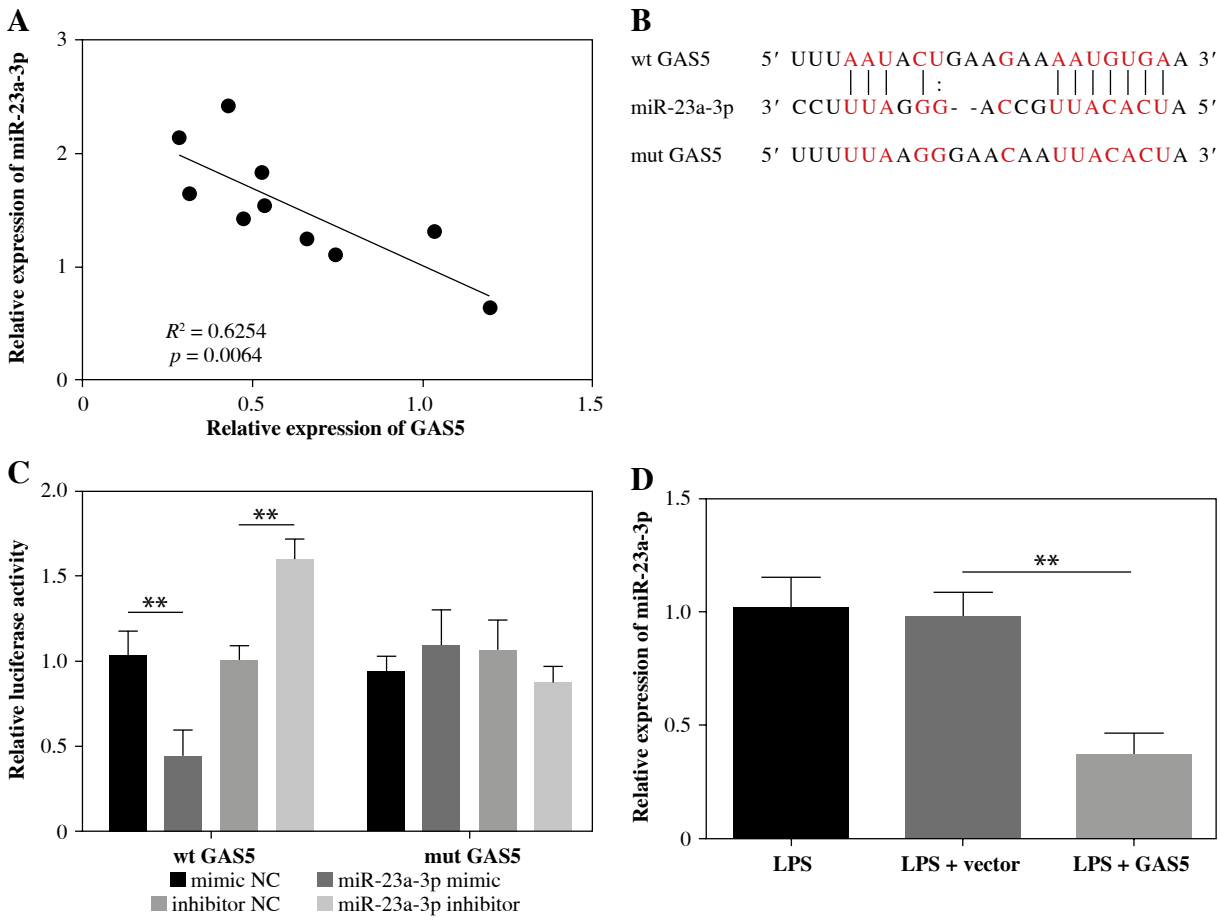

Fig. 3. *GAS5* targets miR-23a-3p. **A**) The correlation between *GAS5* expression and miR-23a-3p expression in inflammatory bowel disease (IBD) patients was determined by Pearson's correlation coefficient. **B**) The binding location between *GAS5* and miR-23a-3p was predicted using the StarBase database. **C**) Binding between miR-23a-3p and *GAS5* was confirmed by the luciferase activity assay. **D**) LPS-treated human fetal colon (FHC) cells were delivered with vectors expressing *GAS5* or empty vectors. miR-23a-3p expression was determined by RT-qPCR

impeded apoptosis, and inhibited inflammation of FHC cells exposed to LPS. These findings underscore the potential of *GAS5* as a promising therapeutic target for IBD and provide a reference for developing novel therapeutic interventions targeting *GAS5*.

GAS5 has been proven to regulate the development of human diseases through acting as a miRNA sponge in some pathological conditions. For instance, *GAS5* exacerbated myocardial depression in septic mice *via* serving as a miR-449b sponge and subsequently repressing high-mobility group box 1 expression [28]. *GAS5* also attenuated cell viability and inhibited cell autophagy by targeting miR-23a/*ATG3* expression [29]. Furthermore, Gao *et al.* observed that *GAS5* targeted miR-23a-3p and Toll-like receptor 4 in septic conditions [30]. Liu *et al.* also found that *GAS5* suppressed the proliferation and invasion of osteosarcoma cells *via* targeting miR-23a-3p and the PI3K/AKT pathway [31]. Here, our experiments showed that *GAS5* bound with miR-23a-3p and decreased its level in LPS-treated FHC cells. In addition, miR-23a-3p was increased in IBD pathological conditions. Blockade of miR-23a-3p enhanced the viability of LPS-treated FHC cells and impeded their apoptosis and inflammation. Whether other factors are involved in the *GAS5*-miR-23a-3p network during IBD development warrants further exploration.

Intestinal epithelial cell apoptosis is a major pathological feature of IBD. It is important for IBD improvement to inhibit the abnormal apoptosis in intestinal epithelial cells [32]. BVES, also named as POPDC1, is expressed in cardiac tissue, skeletal tissue, and the gastrointestinal epithelium, contributing to the maintenance of epithelial integrity [33]. BVES is required for maintaining colonic epithelial integrity in colitis [17], and mice lacking BVES exhibited worse intestinal injury and inflammation [16]. Additionally,

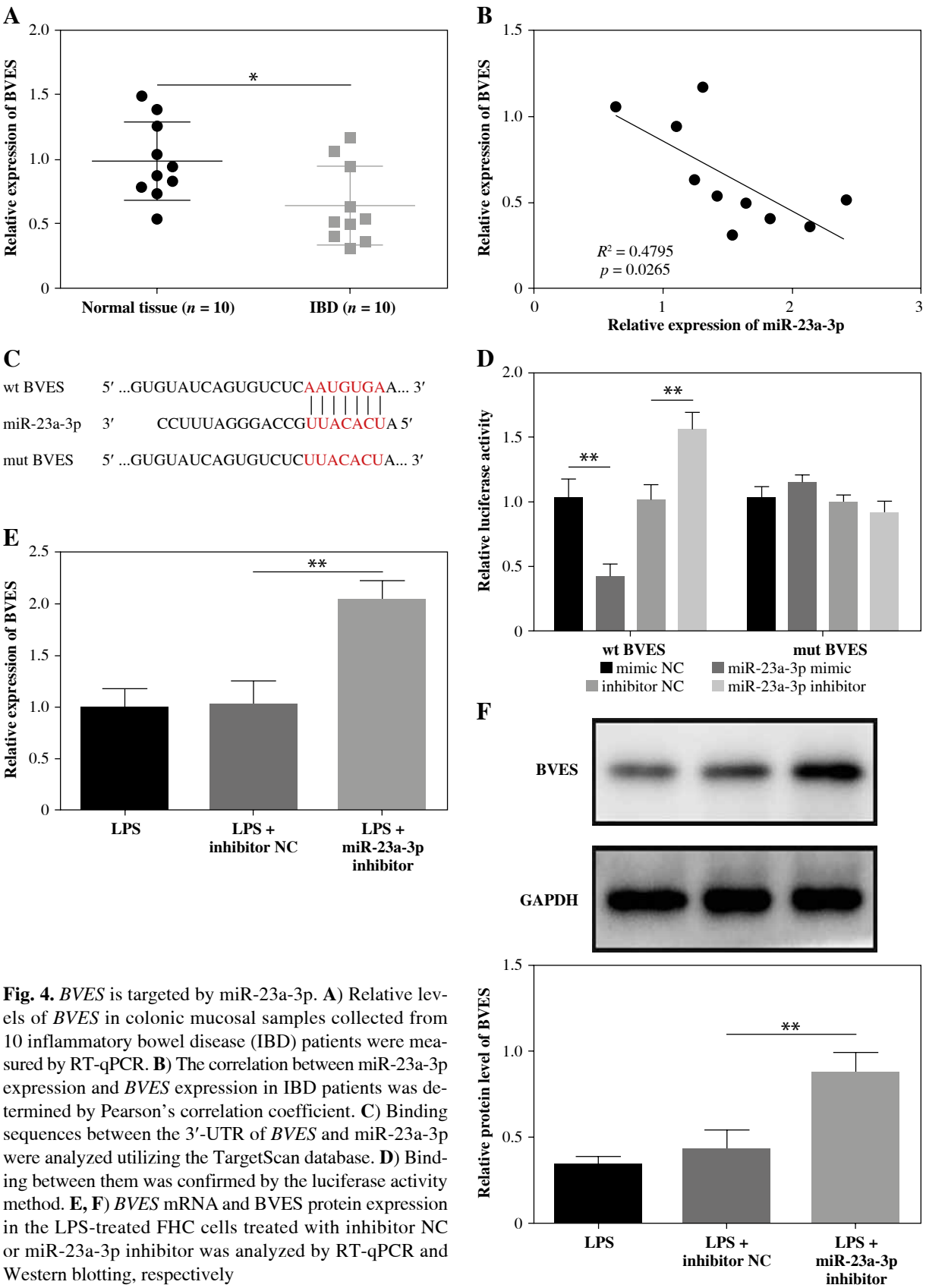

Fig. 4. *BVES* is targeted by miR-23a-3p. **A**) Relative levels of *BVES* in colonic mucosal samples collected from 10 inflammatory bowel disease (IBD) patients were measured by RT-qPCR. **B**) The correlation between miR-23a-3p expression and *BVES* expression in IBD patients was determined by Pearson's correlation coefficient. **C**) Binding sequences between the 3′-UTR of *BVES* and miR-23a-3p were analyzed utilizing the TargetScan database. **D**) Binding between them was confirmed by the luciferase activity method. **E, F**) *BVES* mRNA and BVES protein expression in the LPS-treated FHC cells treated with inhibitor NC or miR-23a-3p inhibitor was analyzed by RT-qPCR and Western blotting, respectively

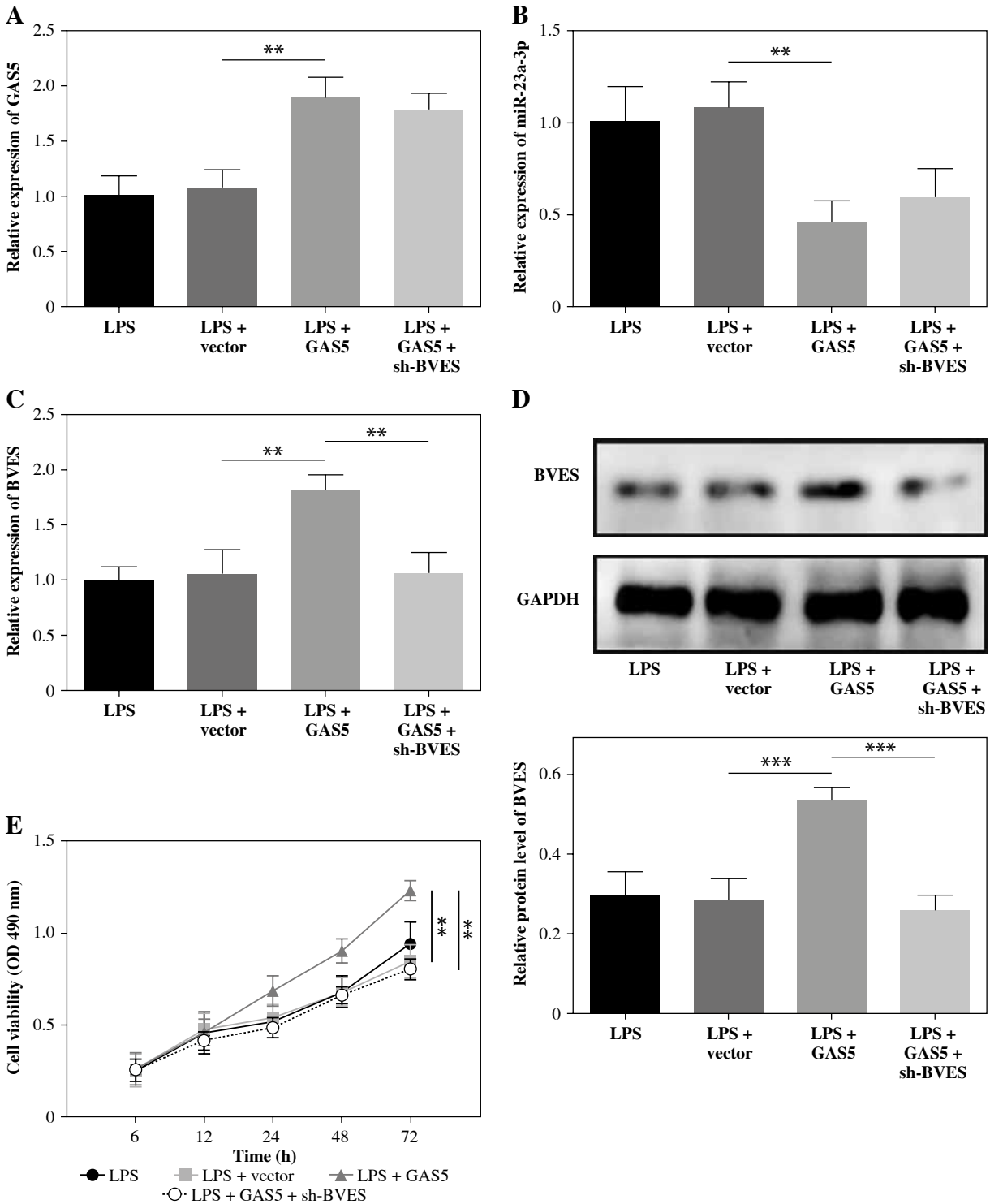

Fig. 5. *GAS5* regulated human fetal colon (FHC) cell viability and apoptosis, and the production of cytokines *via* targeting *BVES*. LPS-treated FHC cells were transfected with vectors expressing *BVES* shRNA and *GAS5*-overexpressing vectors. Levels of **A**) *GAS5*, **B**) miR-23a-3p, and **C**) *BVES* were measured through RT-qPCR. **D**) The protein level of BVES was examined by Western blotting assay. **E**) The MTT method was used to assess the viability of FHC cells

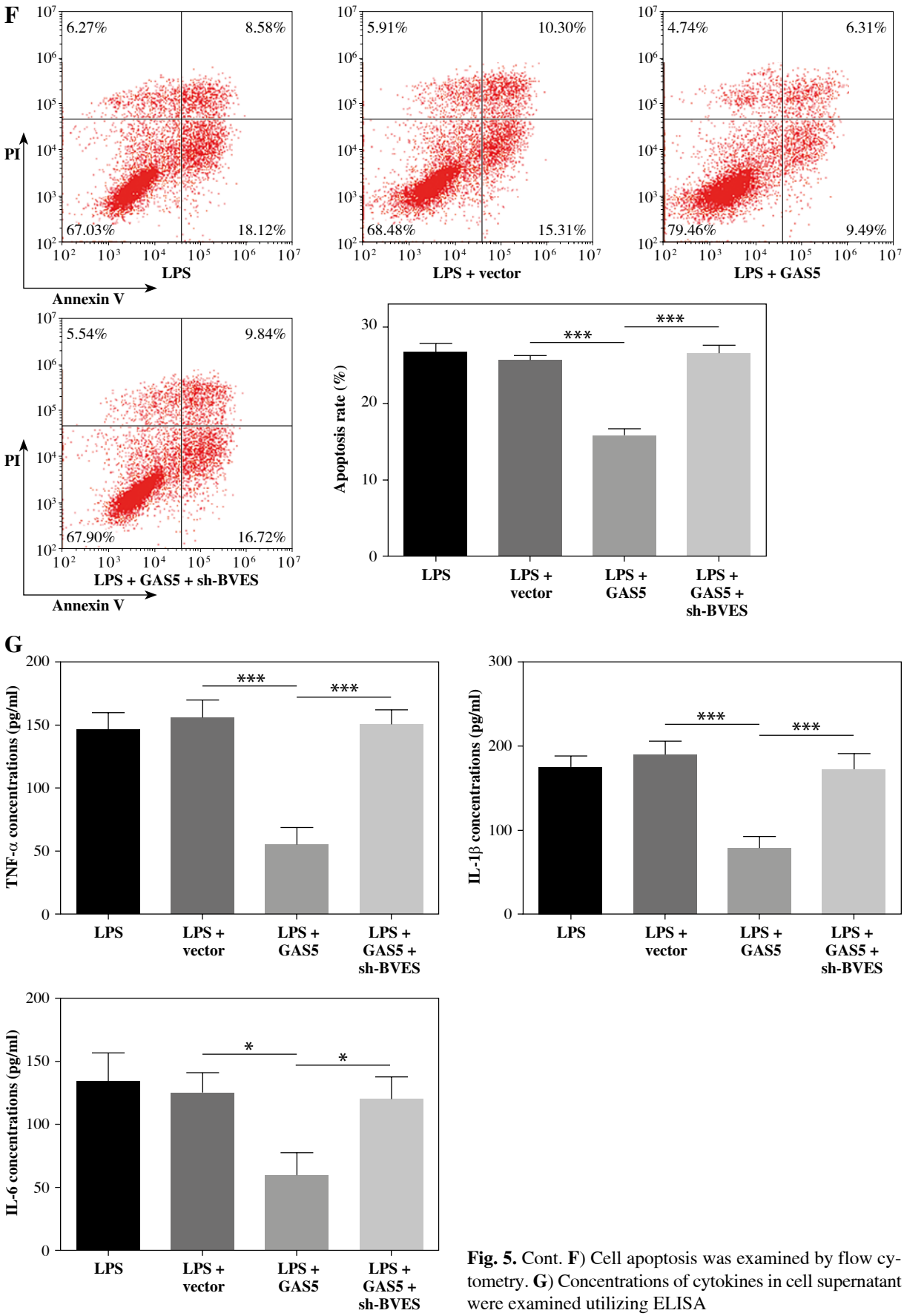

Fig. 5. Cont. **F**) Cell apoptosis was examined by flow cytometry. **G**) Concentrations of cytokines in cell supernatant were examined utilizing ELISA

BVES regulated intestinal stem cell programs and intestinal crypt viability after radiation [34]. *BVES* has also been shown to be regulated by *BVES* antisense RNA 1, inhibiting the proliferation, migration, and invasion of colon adenocarcinoma cells [35]. Here, our results indicated that *BVES* was decreased in the colonic mucosal samples of IBD patients, and it was a downstream gene of miR-23a-3p. Silencing of *BVES* notably reversed the impact of *GAS5* on the proliferative process, inflammation, and apoptosis of FHC cells exposed to LPS. The identification of the regulatory roles of *GAS5* and its downstream targets suggests their potential utility as biomarkers for developing diagnostic tools aimed at early detection and personalized management of IBD.

There are some limitations of the present study. First, we only explored the effects of *GAS5*, miR-23a-3p, and *BVES*, and their interactions *in vitro*. These findings need to be validated with a larger sample size and in animal models. Second, the functions of the "Full-length" (FL) and "Clone 2" (C2) variants of *GAS5* were not explored in this study. FL has been shown to considerably enhance cell proliferation by rescuing cell cycle arrest, while C2 is responsible for driving apoptosis [36]. Further investigations on the distinct roles of the two variants are warranted.

Conclusions

In conclusion, our findings highlight the pivotal role of *GAS5* in enhancing the viability and suppressing apoptosis and inflammation in FHC cells through upregulation of *BVES via* miR-23a-3p binding. These results may provide a foundation for future research aimed at translating these findings into clinical applications that could improve outcomes for patients with IBD.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

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Disclosures

The study protocol received approval by the Ethics Committee of the First Affiliated Hospital, Anhui University of Science and Technology and adhere to the principles outlined in the Declaration of Helsinki (approval number: 2023-KY-150-001). Written informed consent was collected from all participants.

The authors declare no conflict of interest.

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