

Expression of interleukin-22/STAT3 signaling pathway in ulcerative colitis and related carcinogenesis

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by Western blotting.

RESULTS: Patients with active UC had significantly more IL-22, IL-23, IL-22R1 and p-STAT3-positive cells than the patients with inactive UC and normal controls. Furthermore, IL-22 and related proteins were closely related to the severity of the colitis. The expression of IL-22 and IL-22R1 in the tissue of initial UC was stronger than in that of chronic UC, whereas the expression of p-STAT3 was significantly increased in chronic UC tissues. In dysplasia tissues, the expression level of IL-22 and related proteins was higher compared with controls. Mouse colitis model showed that expression of IL-22, IL-22R1 and IL-23 was increased with time, p-STAT3 and the downstream gene were also remarkably upregulated.

CONCLUSION: IL-22/STAT3 signaling pathway may be related to UC and UC-induced carcinogenesis and IL-22 can be used as a biomarker in judging the severity of UC.

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Abstract

AIM: To investigate the expression of interleukin (IL)-22 and its related proteins in biopsy specimens from patients with ulcerative colitis (UC) and UC-related carcinogenesis.

METHODS: Biopsy specimens were obtained from patients with inactive ($n = 10$), mild-to-moderately active ($n = 30$), severely active ($n = 34$), initial ($n = 30$), and chronic UC ($n = 44$), as well as UC patients with dysplasia ($n = 10$). Specimens from patients without colonic abnormalities ($n = 20$) served as controls. Chronic colitis in experimental mice was induced by 2.5% dextran sodium sulfate. The expression levels of IL-22, IL-23, IL-22R1 and phosphorylated STAT3 (p-STAT3) were determined by immunohistochemistry. Bcl-2, cyclin D1 and survivin expression was detected

Key words: Ulcerative colitis; Ulcerative colitis-related carcinogenesis; Interleukin-22; Interleukin-22R1; STAT3

Core tip: This study investigates the expression of interleukin (IL)-22, IL-22R1, IL-23, and STAT3 in ulcerative colitis (UC) and UC-related carcinogenesis (UC-CRC) tissues from human and mouse. The results showed that IL-22 and related proteins were closely related to the severity of colitis, and the expression level of IL-22 and related proteins was higher in dysplasia tissues. IL-22/STAT3 signaling pathway was related to UC and UC-CRC. IL-22 can be used as a biomarker for determining the severity of UC and as an interesting therapeutic target in active UC and UC-CRC.

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INTRODUCTION

Ulcerative colitis (UC) is a subtype of chronic inflammatory bowel disease (IBD) of the large intestine. The disease is characterized by a dysregulated mucosal immune response. This aberrant immune response leads to the secretion of harmful cytokines that destroy the gastrointestinal tract epithelium, thereby causing further inflammation.

Several inflammatory cytokines have been associated with IBD, including the interleukins IL-1 and IL-6, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ . Among the inflammatory cytokines implicated in IBD pathogenesis, much interest has been focused on the recently-identified cytokine IL-22. IL-22 is a member of the IL-10 subfamily; its production is highly dependent on IL-23 in T-helper 17 (Th17)^[1-3], Th1^[4,5], NK-22^[6,7], and CD11c⁺^[8] cells. IL-22 is expressed by the novel Th22 cell lineages^[9] and innate lymphoid cells (ILCs)^[10]. IL-22-producing ILCs in humans are responsive to IL-23 signaling, as potentially important mediators of IBDs^[10]. Th22 and Th17 cells may be implicated in the pathogenesis of several chronic inflammatory and autoimmune diseases such as IBD, psoriasis, ankylosing spondylitis, and rheumatoid arthritis^[11-16].

IL-22 signaling is established when the cytokine binds to a heterodimeric receptor complex of IL-22R1 and IL-10R2. Given that IL-10R2 is a ubiquitous protein, cellular IL-22 responsiveness is mainly determined by IL-22R1 expression. IL-22R1 is specifically expressed in nonleukocytic cells such as those of the pancreas, skin, kidney, liver, and colon. IL-22R1 expression is detectable in epithelial cells of these organs, but not in their immune cells^[17-20]. Therefore, IL-22 is unique among the cytokines because it cannot mediate autocrine or paracrine functions among leukocytes. Instead, IL-22 transmits information between leukocytes and the nonleukocytic cell compartment.

The STAT3 pathway for transcription activation appears to be a major mode of IL-22 signal transduction. Activated STAT3 is translocated from the cytoplasm to the nucleus, where it regulates genes involved in cell apoptosis, proliferation, migration, and survival. STAT3 has important functions in several autoimmune diseases. IL-22 mediates IL-23-induced acanthosis and dermal inflammation in psoriasis and IBD through the activation of STAT3^[21]. When activated by IL-22, STAT3 can aggravate colitis by promoting the expression of inflammatory factors such as IL-8, IFN- γ , and the matrix metalloproteinases (MMPs)^[22,23]. Previous studies investigated the role of IL-22 in UC and UC-related carcinogenesis (UC-CRC)^[24-26]. These studies revealed that the IL-22/

STAT3 pathway is involved in UC pathophysiology and carcinogenesis through the activity of inducible nitric oxide synthase (iNOS), DMBT1, and REG α ^[24-26]. However, the IL-22-induced phosphorylated STAT3 (p-STAT3) was considered a defense mechanism because it enhanced mucus production and goblet cell replacement in mouse models for acute colitis and wound healing^[27-29]. Thus, the role of the IL-22/STAT3 signaling pathway in UC remains unclear. The current study investigates the significance of IL-22 and the IL-22/STAT3 signaling pathway in UC and UC-CRC, as well as its value as a therapeutic target for both diseases.

MATERIALS AND METHODS

Patients and tissue samples

Colon biopsy *via* endoscopy was performed on 74 patients with UC, including 31 females and 43 males (median age, 45.9 years; range, 17-87 years). The controls included 6 females and 14 males (mean age, 45.6 years; range, 33-55 years). All samples were obtained at the First Affiliated Hospital of Nanjing Medical University from 2009 to 2011.

This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. Written informed consent was obtained from each patient. The diagnosis of UC was based on clinical, endoscopic, and histological findings. The patient characteristics and histological data are summarized in Table 1.

Experimental mouse models of UC

The mouse experiments were conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the United States National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Nanjing Medical University. Male ICR mice were purchased and maintained at the Centre of Animal Facility, Nanjing Medical University. The mice were sacrificed by cervical dislocation. All efforts were made to minimize suffering. Chronic colitis was induced by administering 2.5% dextran sodium sulfate (DSS; ICN Biomedicals Inc., Irvine, CA, United States) to the drinking water in three seven-day cycles, which had a five-day recovery period after each cycle. During the recovery period, mice drank only normal water. The age-matched control mice received only normal drinking water throughout the entire study.

Immunohistochemistry

All the tissues were fixed overnight in 4% paraformaldehyde at 4 °C, processed, and cut into 5 μ m-thick sections. The sections were then deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was blocked by 3% H₂O₂ for 10 min at room temperature. Antigen retrieval was performed by 15 min of boiling in the pre-heated buffer (10 mmol/L of sodium citrate, pH = 6.0)

Table 1 Clinical characteristics of ulcerative colitis patients

	Normal control <i>n</i> = 20	Histological activity of UC			Types of UC		UC with dysplasia <i>n</i> = 10
		Inactive <i>n</i> = 10	Mild-moderate <i>n</i> = 30	Severe <i>n</i> = 34	Initial <i>n</i> = 30	Chronic <i>n</i> = 44	
Gender (<i>n</i>)							
Male	14	5	16	22	16	27	6
Female	6	5	14	12	14	17	4
Mean age ³ , yr	40.6 ± 12.5	41.2 ± 13.3	44.7 ± 13.2	43.2 ± 17.1	42.8 ± 17.3	46.5 ± 13.6	44.7 ± 16.6
Mean duration of disease ³ , yr	-	6.14 ± 4.2	4.3 ± 2.3	3.7 ± 3.1	0.8 ± 0.6	5.1 ± 0.6 ¹	10.5 ± 1.4 ²
Extent of disease (<i>n</i>)							
Extensive colitis	-	0	8	19	8	19	3
Left-side colitis	-	4	13	9	13	13	4
Proctitis	-	6	9	6	9	12	3
Treatment (<i>n</i>)							
Aminosalicylates	-	8	25	20	16	35	5
Corticosteroids	-	4	5	18	4	15	3
Immunosuppressive agent	-	0	0	1	0	1	0
Biological agent	-	0	0	0	0	0	0
None	-	3	2	0	10	5	2

¹The mean duration of disease in the chronic group (5.1 ± 0.6 years) was significantly longer than in the initial group (0.8 ± 0.6 years; $P < 0.001$); ²The mean duration of disease in the ulcerative colitis (UC) with dysplasia group (10.5 ± 1.4 years) was significantly longer than in other groups ($P < 0.05$); ³Data are expressed as mean ± SD.

at 200 W in a microwave. The slides were transferred to a humidifier and blocked by incubating in 5% normal goat serum at room temperature for 1 h. The polyclonal rabbit antibodies used in this study included anti-p-STAT3 tyrosine 727, anti-IL-22, anti-IL-22R1, and anti-IL-23 (ab30647, ab18499, ab5984 and ab115759, respectively); these antibodies were diluted in 5% normal goat serum at ratios of 1:200, 1:200, 1:200 and 1:400, respectively. The sections were incubated in the respective antibodies overnight at 4 °C. The slides were subsequently incubated in the secondary antibody goat anti-rabbit IgG-biotin (B8895; Sigma-Aldrich, St. Louis, MO, United States) at room temperature for 40 min. The sections were incubated in the ABC-peroxidase solution (Ultrasensitive™ S-P kit, kit 9719; Maixin-Bio, China) for 30 min at room temperature, followed by counterstaining with hematoxylin. The immunohistochemistry (IHC) results were analyzed using Image Pro-Plus.

Western blotting analysis

Proteins were extracted from the mouse tissues and quantified using a commercial protein assay (Bio-Rad Laboratories, CA, United States). The protein samples (30 µg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. Immunoblot analysis was conducted using antibodies against total STAT3, p-STAT3 (S727), Bcl-2, cyclin D1, and survivin (Abcam Inc, MA, United States). The results were visualized using the chemiluminescent Pierce ECL Substrate Western blotting detection system (Thermo Scientific, IL, United States) and exposure to autoradiography film (Kodak XAR film).

Statistical analysis

The results were expressed as the mean ± SD. The two

groups were compared using the Student's *t* test or the Mann-Whitney *U* test, as appropriate. All statistical analyses were performed using the SPSS statistical software (version 13.0). $P < 0.05$ was considered to be statistically significant.

RESULTS

Patient characteristics

As shown in Table 1, we collected biopsy specimens from 20 healthy controls, 10 patients with inactive UC, 64 patients with active UC (including 30 patients with mild-to-moderate active UC and 34 patients with severely active UC), 30 patients with initial UC attacks, 44 patients with chronic UC, and 10 UC patients with dysplasia. The duration of disease was significantly longer in the groups with chronic UC and UC with dysplasia than in the other groups ($P < 0.05$). No other significant differences were observed among the other groups, regardless of the treatment used.

IL-22 expression in patients with different degrees of inflammation

Immunohistochemical analysis showed that the IL-22 protein was mainly expressed in inflammatory cells of the colonic lamina propria, but not in the normal controls (Figure 1A). Tissues with mild-moderate and severe UC had significantly higher expression levels than those with inactive UC and normal colon tissues (mild-moderate *vs* normal, $P < 0.001$; mild-moderate *vs* inactive, $P = 0.02$, $P < 0.05$; severe *vs* normal, $P < 0.001$; severe *vs* inactive, $P < 0.001$; Figure 2A). Moreover, significantly more IL-22-positive cells were present in tissues of severe UC than in those of mild-moderate UC (severe *vs* mild-moderate, $P < 0.001$; Figure 2A). The results indicate that

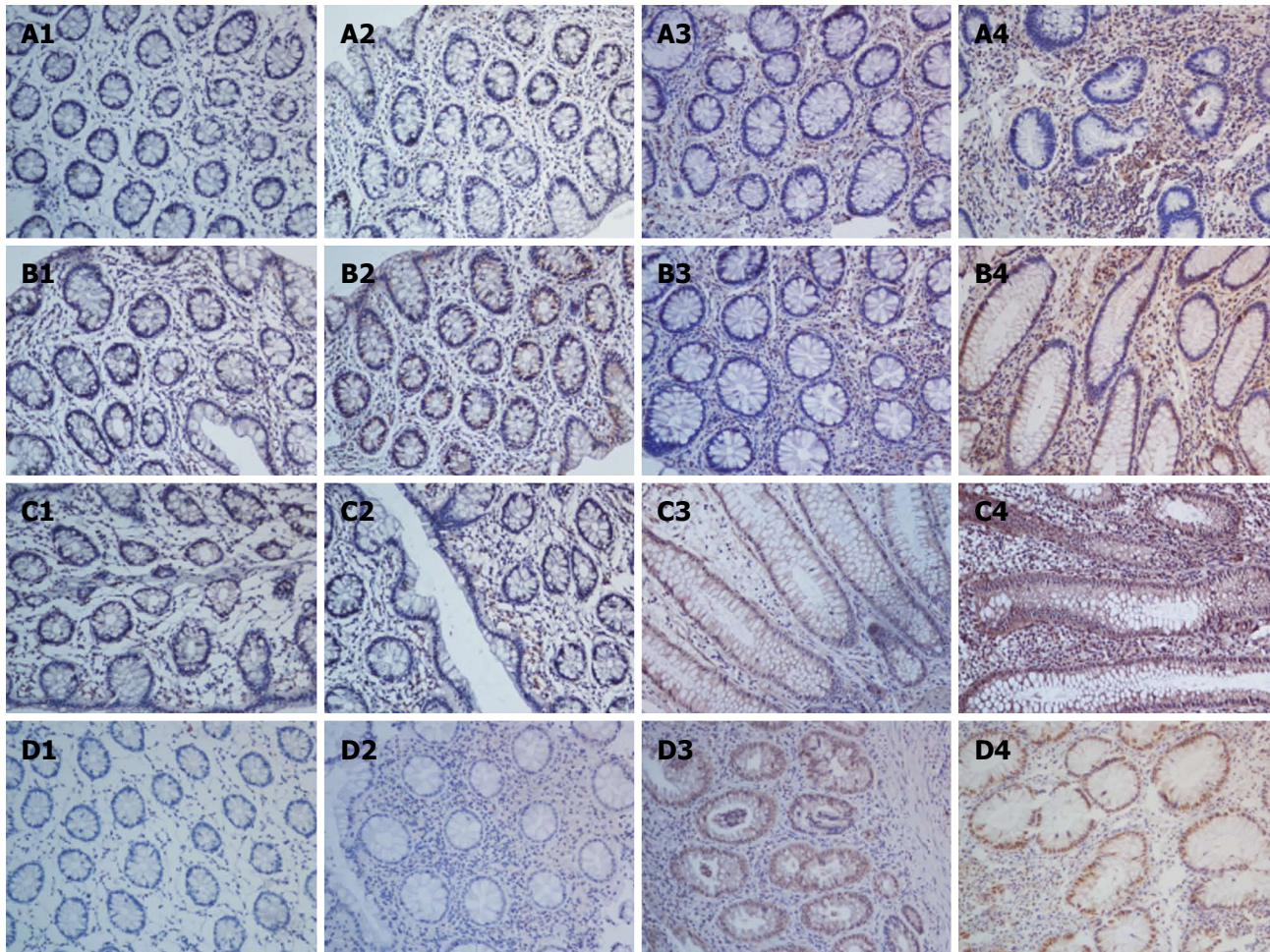


Figure 1 Expression and distribution of interleukin-22 and its related proteins in colonic biopsy specimens as analyzed by immunohistochemistry ($\times 200$). A1-A4: Expression and distribution of interleukin (IL)-22 in colonic biopsy specimens from the control, inactive ulcerative colitis (UC), mild-moderate UC, and severe UC tissues, respectively; B1-B4: The expression and distribution of IL-23 in colonic biopsy specimens from the control, inactive UC, mild-moderate UC, and severe UC tissues; C1-C4: The expression and distribution of IL-22R1 in colonic biopsy specimens from the control, inactive UC, mild-moderate UC, and severe UC tissues; D1-D4: The expression and distribution of p-STAT3 (S727) in colonic biopsy specimens from the control, inactive UC, mild-moderate UC, and severe UC tissues.

the expression of IL-22 was related to UC severity.

Expression of IL-22-related proteins in patients with different degrees of inflammation

Previous studies have demonstrated that IL-22 production is highly dependent on IL-23 in Th17, NK-22, and lymphoid tissue-inducer cells^[1-3,6,7]. Thus, we analyzed IL-23 expression in UC tissues using IHC. The results indicated that IL-23 was significantly highly upregulated in active UC, as compared with inactive UC and the controls (mild-moderate *vs* normal, $P < 0.001$; mild-moderate *vs* inactive, $P < 0.001$; severe *vs* normal, $P < 0.001$; severe *vs* inactive, $P < 0.001$; Figure 2B). Similarly, the IL-23 expression was stronger with severe UC than with mild-moderate UC (severe *vs* mild-moderate, $P = 0.01$, $P < 0.05$; Figure 2B). The positive region of IL-23 in UC was mostly confined to the intestinal epithelial cells (IECs) and inflammatory cells of the colonic lamina propria (Figure 1B).

We identified IL-22R1, another key molecule that is necessary for signal transmission. IL-22R1 was mainly

localized in the IECs (Figure 1C). IL-22R1 was overexpressed in active UC (mild-moderate *vs* normal, $P < 0.001$; mild-moderate *vs* inactive, $P = 0.0002$, $P < 0.001$; severe *vs* normal, $P < 0.001$; severe *vs* inactive, $P = 0.0002$, $P < 0.001$; severe *vs* mild-moderate, $P = 0.007$, $P < 0.01$; Figure 2C).

Based on the downstream effects of IL-22, the activation of STAT3 was determined by staining with p-STAT3 at the S727 residue. IHC analysis showed that in the colon tissues resected from patients with UC, the p-STAT3 (S727) protein was mainly expressed in the nucleus of epithelial cells (Figure 1D). Its expression was significantly upregulated in the active UC tissues, particularly in severe UC (severe *vs* normal, $P < 0.001$; severe *vs* inactive, $P < 0.001$; severe *vs* mild-moderate, $P = 0.0045$, $P < 0.01$; mild-moderate *vs* normal, $P < 0.001$; mild-moderate *vs* inactive, $P = 0.0017$, $P < 0.01$; Figure 2D).

IL-22 expression in patients with different types of UC

According to the clinical diagnostic standards, we classified UC into two types: initial and chronic UC. IHC

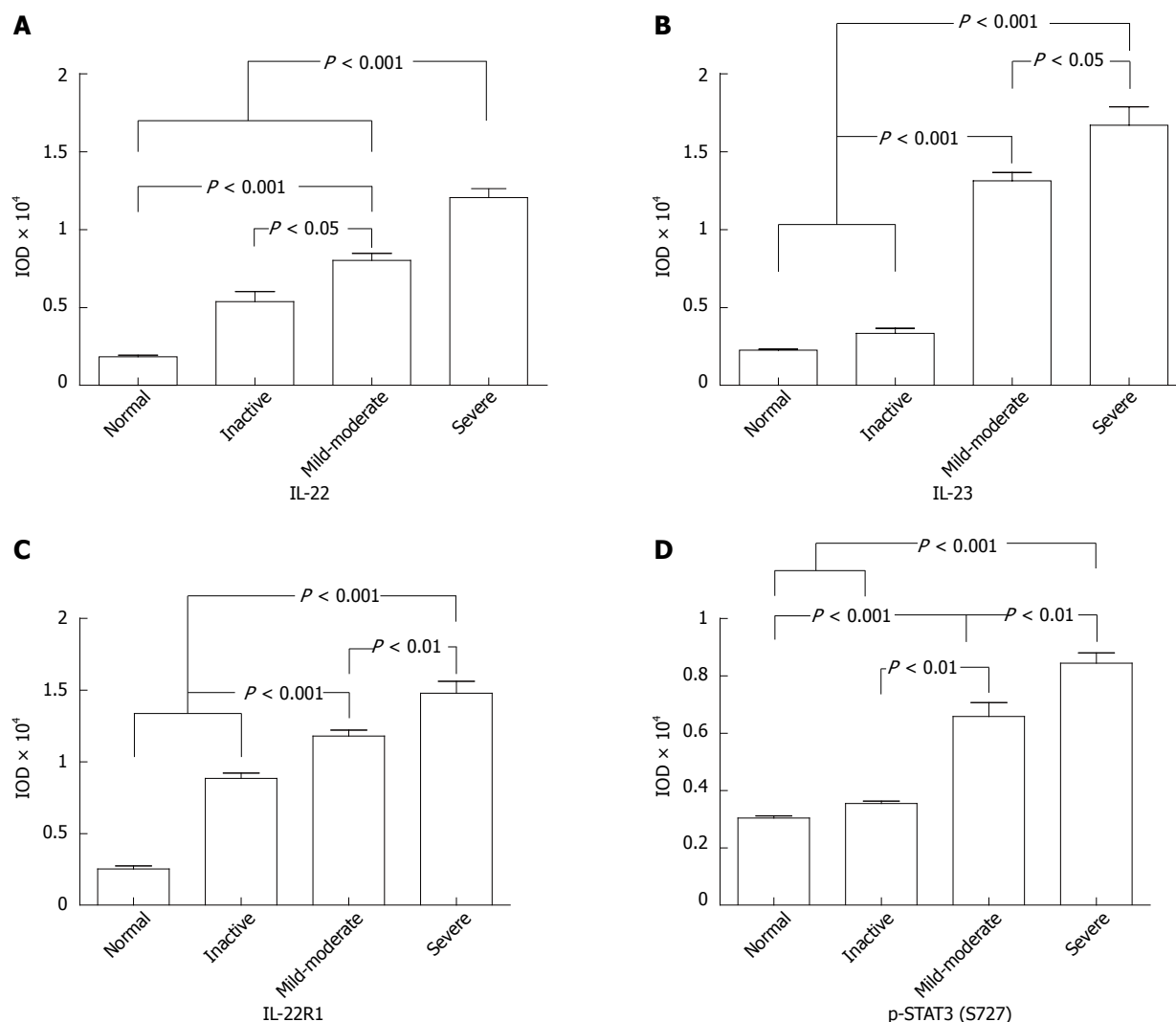


Figure 2 Statistical evaluation of interleukin-22 and its related proteins in human colonic biopsy specimens from the controls and patients with inactive, mild-moderate, and severe ulcerative colitis. The average integrated optical density (IOD) was obtained by analyzing five random fields for each slide, which were evaluated using Image-Pro Plus software (v. 5.0), for the immunohistochemistry staining of interleukin (IL)-22 (A), IL-23 (B), IL-22R1 (C), and p-STAT3 (S727) (D) in human colonic biopsy specimens. The expression levels of IL-22 (A), IL-23 (B), IL-22R1 (C), and p-STAT3 (D) were positively correlated with colitis severity.

indicated that IL-22 was significantly upregulated in the initial UC tissues than in the chronic UC tissues (initial *vs* chronic, $P = 0.0077$, $P < 0.01$; Figures 3A and 4A).

IL-22-related protein expression in patients with different types of UC

Similar to IL-22, IL-22R1 was more strongly expressed in the tissues of initial UC than in those of chronic UC (initial *vs* chronic, $P < 0.001$; Figures 3C and 4C). By contrast, the number of p-STAT3-positive cells was significantly higher in chronic UC tissues than in initial UC (chronic *vs* initial, $P = 0.03$, $P < 0.05$; Figures 3D and 4D). However, no significant differences were detected in terms of the IL-23 expression in these two groups (Figures 3B and 4B).

IL-22 expression in patients with UC-CRC (dysplasia)

A positive correlation exists between the IL-22-positive cells and the severity of colitis in patients with UC. We investigated IL-22 expression in biopsy specimens from

patients with UC-CRC (dysplasia) and analyzed the IL-22 levels in UC with dysplasia (Figure 5A). Significantly more IL-22-positive cells were observed in the dysplasia group than in the inflammatory group ($P = 0.02$; Figure 6A).

Expression of IL-22-related proteins in patients with UC-CRC (dysplasia)

Given that IL-22 was highly upregulated in UC tissue with dysplasia, we studied the expression of the receptor IL-22R1, its upstream IL-23, and its downstream p-STAT3 (S727) in UC tissues with dysplasia, as compared with active and inactive UC tissues. The expression levels of IL-22R1, IL-23, and p-STAT3 were significantly higher in UC tissues with dysplasia than in the control group (IL-22R1: dysplasia *vs* active, $P = 0.02$; IL-23: dysplasia *vs* active, $P = 0.01$; p-STAT3: dysplasia *vs* active, $P = 0.02$; Figure 6B-D). The increased expression was strictly found at the dysplastic tissues of the patients (Figure 5B-D).

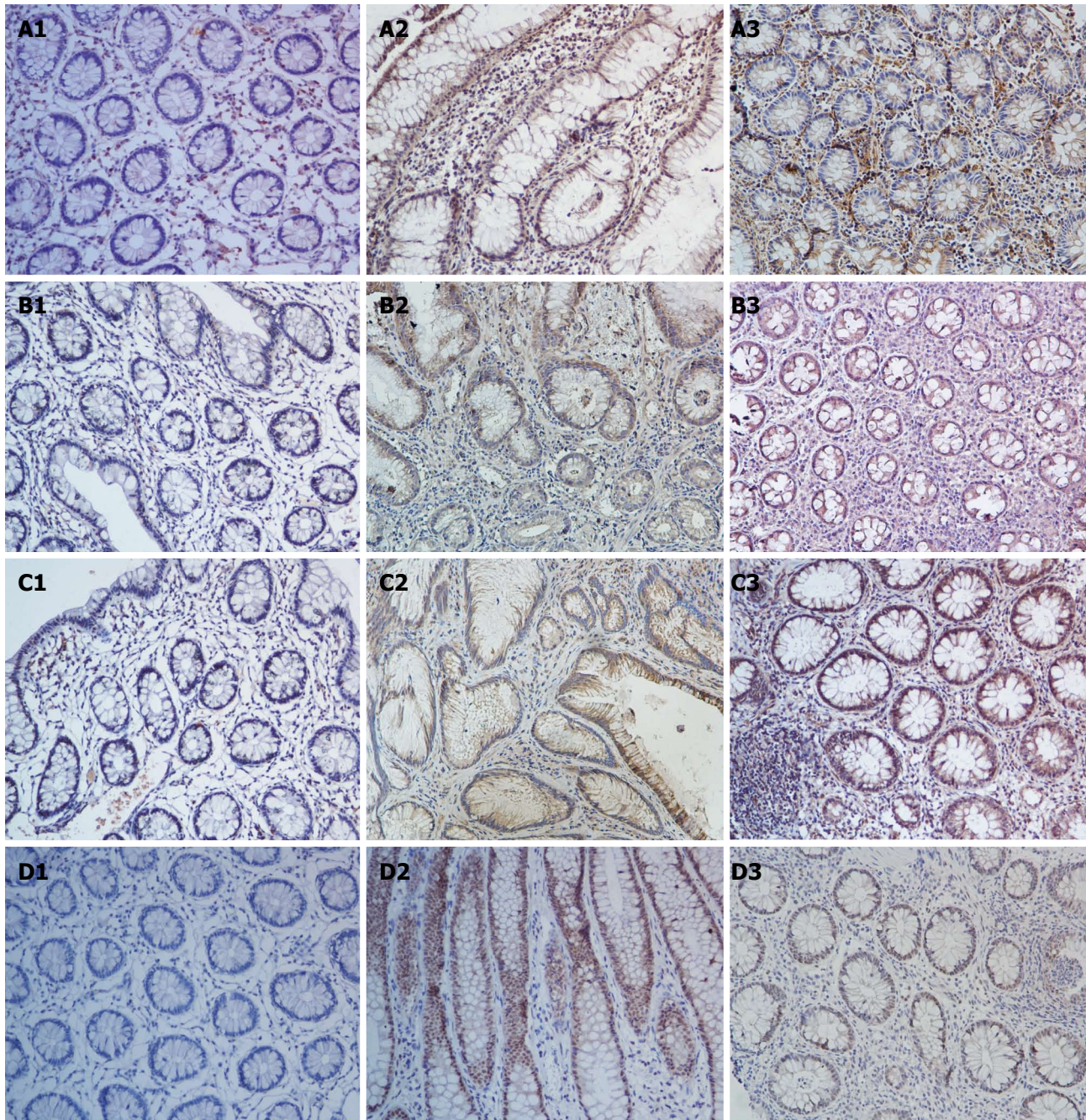


Figure 3 Expression and distribution of interleukin-22 and related proteins in human colonic tissues from controls and patients with chronic and initial ulcerative colitis as detected by immunohistochemistry ($\times 200$). A1-A3: Expression and distribution of interleukin (IL)-22 in ulcerative colitis (UC) tissues from the control, chronic UC, and initial UC groups; B1-B3: Expression and distribution of IL-23 in UC tissues from the control, chronic, and initial UC groups; C1-C3: Expression and distribution of IL-22R1 in UC tissues from the control, chronic, and initial UC groups; D1-D3: Expression and distribution of p-STAT3 in UC tissues from the chronic and initial UC groups.

Expression of IL-22 and its related proteins in DSS-induced mouse models of chronic colitis

We induced experimental colitis by treating mice with DSS to study the role of IL-22 and its related proteins in the disease. Dynamic IL-22, IL-23, and IL-22R1 expression levels were investigated by IHC. p-STAT3 activity and its downstream gene expression were confirmed by Western blotting analysis at different time points (at days 40, 80, and 120). The expression levels of IL-22, IL-22R1, and IL-23 were increased with time (Figure 7A).

STAT3 activation and the activity of its downstream cell proliferation-related genes, such as Bcl-2, cyclin D1, and survivin, were also investigated. All these genes had sustained expression over time (Figure 7B).

DISCUSSION

IBDs such as Crohn's disease and ulcerative colitis are chronic inflammatory disorders of the gastrointestinal tract. Although their etiology is not completely under-

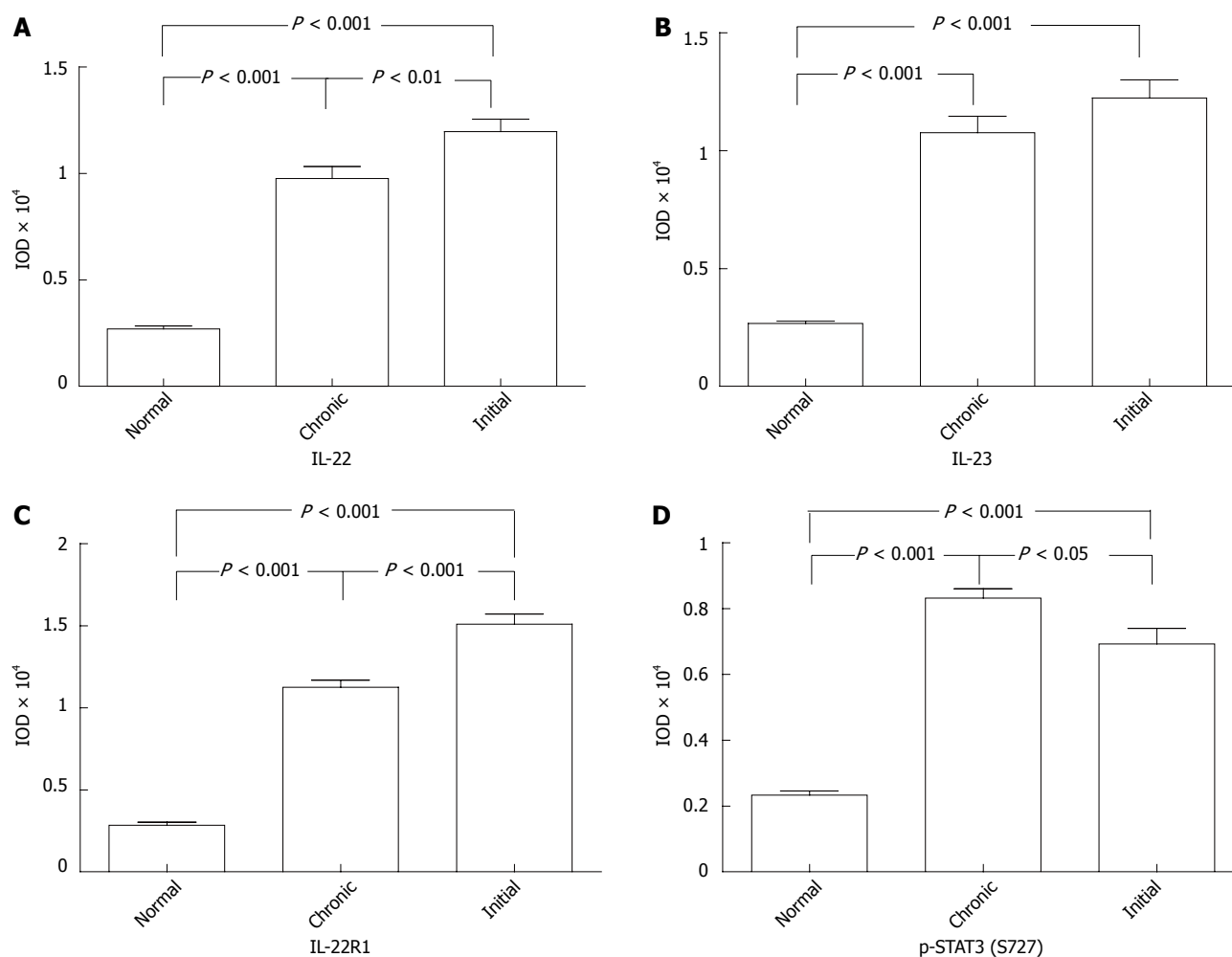


Figure 4 Statistical evaluation of interleukin-22 and its related proteins in human colonic biopsy specimens from controls and patients with chronic and initial ulcerative colitis. The average integrated optical density (IOD) for the immunohistochemistry staining of interleukin (IL)-22 (A), IL-23 (B), IL-22R1 (C), and p-STAT3 (S727) (D) in colonic tissues with initial and chronic ulcerative colitis (UC). Significantly higher expression levels of IL-22 and IL-22R1 were observed in the initial UC group as compared with the chronic UC group. p-STAT3 was highly expressed in the chronic UC group.

stood, initiation and aggravation of the inflammatory process seem to be related to a massive local mucosal immune response. IL-22 belongs to the IL-10 family of cytokines; it has recently been shown to be preferentially expressed by Th17 and Th22 cells. These cells have been identified in the pathogenesis of certain chronic inflammatory diseases, including colitis, psoriasis, and rheumatoid arthritis. IL-22 targets innate immune pathways because of the restricted expression of IL-22 receptors on nonleukocytic cells, such as epithelial cells, keratinocytes, and hepatocytes; however, it does not recognize T- or B-cells^[17-20]. Studies using genetically-engineered mice have demonstrated that epithelial STAT3 activation in DSS-induced colitis is dependent on IL-22, rather than on IL-6. Both IL-22 and STAT3 activation in epithelial cells is important for wound-healing, as demonstrated by *in vivo* experiments^[27]. Sugimoto *et al.*^[28] found that the IL-22/STAT3 pathway contributes to the rapid amelioration of local intestinal inflammation by enhancing the production of membrane-bound mucins (Muc1, -3, -10, and -13) in a mouse model of acute colitis. However, IL-22 is also considered an inflammatory driver in IBD

by acting on human colonic subepithelial myofibroblasts to stimulate secretion of proinflammatory cytokines such as MMPs, IL-1, IL-8, and INF- γ ^[30]. Highly elevated serum levels of IL-22 were correlated with disease severity in patients with Crohn's disease (CD)^[17]. Colitis mouse models have indicated that highly elevated IL-22 expression may directly or indirectly induce inflammation^[31]. Moreover, the IL-22/STAT3 signaling pathway is important in inflammation and carcinogenesis during UC *via* its upregulation of iNOS and MMP production, respectively^[23,24]. Here, we demonstrate that IL-22 contributes to the inflammatory severity of UC and UC-CRC by activating STAT3 in IECs.

The UC microenvironment is composed of IECs, macrophages, immunocytes, and so on. The interactions among these cells involve their secreted cytokines and consist of a positive feedback loop with persistent activation of the STAT3-enabling progression of UC. Similar to IL-23 and IL-22R1, an IL-22 positive feedback loop in the UC microenvironment was demonstrated in this study. Our results indicated the IL-22 overexpression in the inflammatory cells of the colonic lamina propria in

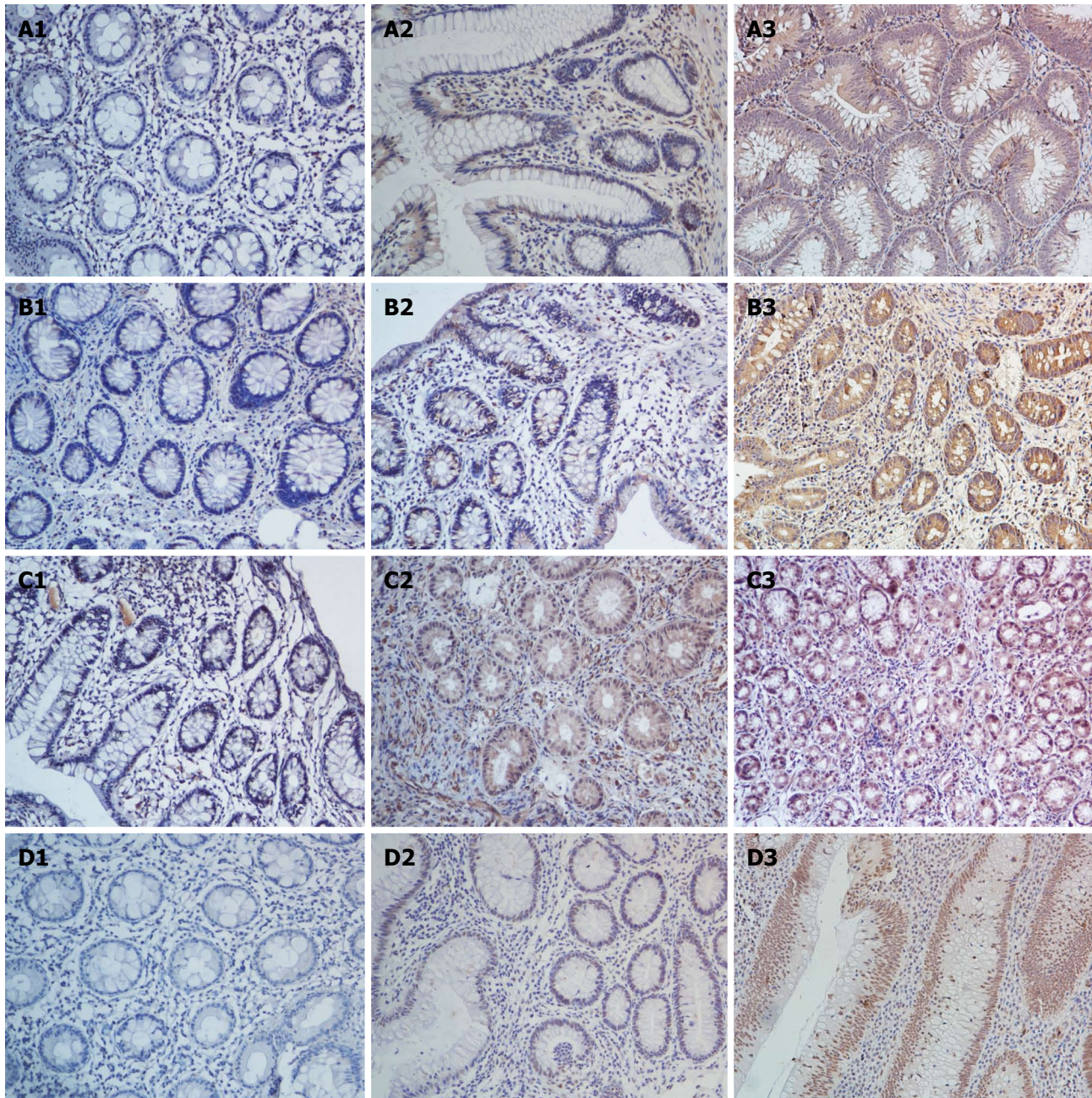


Figure 5 Expression and distribution of interleukin-22 and its related proteins in human colonic tissues from patients with inactive ulcerative colitis, active ulcerative colitis, and ulcerative colitis with dysplasia, as detected by immunohistochemistry ($\times 200$). A1-A3: Expression and distribution of interleukin (IL)-22 in inactive ulcerative colitis (UC), active UC, and UC with dysplasia tissues; B1-B3: Expression and distribution of IL-23 in inactive UC, active UC, and UC with dysplasia tissues; C1-C3: Expression and distribution of IL-22R1 in inactive ulcerative colitis (UC), active UC, and UC with dysplasia tissues; D1-D3: Expression and distribution of p-STAT3 (S727) in inactive UC, active UC, and UC with dysplasia tissues.

UC tissues. Moreover, the sustained activation of STAT3 signaling in IECs was verified. Simultaneously, IL-22R1 expression was enhanced in IECs, thereby ensuring the transmission of the IL-22 signal.

The STAT3-regulated proinflammatory cytokine IL-23^[32] was likewise overexpressed in human UC. IL-23 activates innate immune cells to secrete pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-6, as well as maintains the expansion of Th17 cells that express IL-22^[33].

IL-22 is increased in active Crohn's disease and pro-

motes proinflammatory gene expression^[17]. We demonstrated that IL-22 is more highly expressed in active UC than in inactive UC and the normal control. Thus, the increased IL-22 signaling in active IBD supports the potential of an IL-22 signaling blockade as a therapeutic strategy for IBD. Furthermore, IL-23, IL-22, STAT3, and IL-22R1 are closely related to the colitis severity. Thus, positive feedback loops can further exacerbate inflammation. If left unchecked, these pathways may lead to the chronic immune pathology that is characteristic to IBD. Therefore, IL-22 could be used as a biomarker for deter-

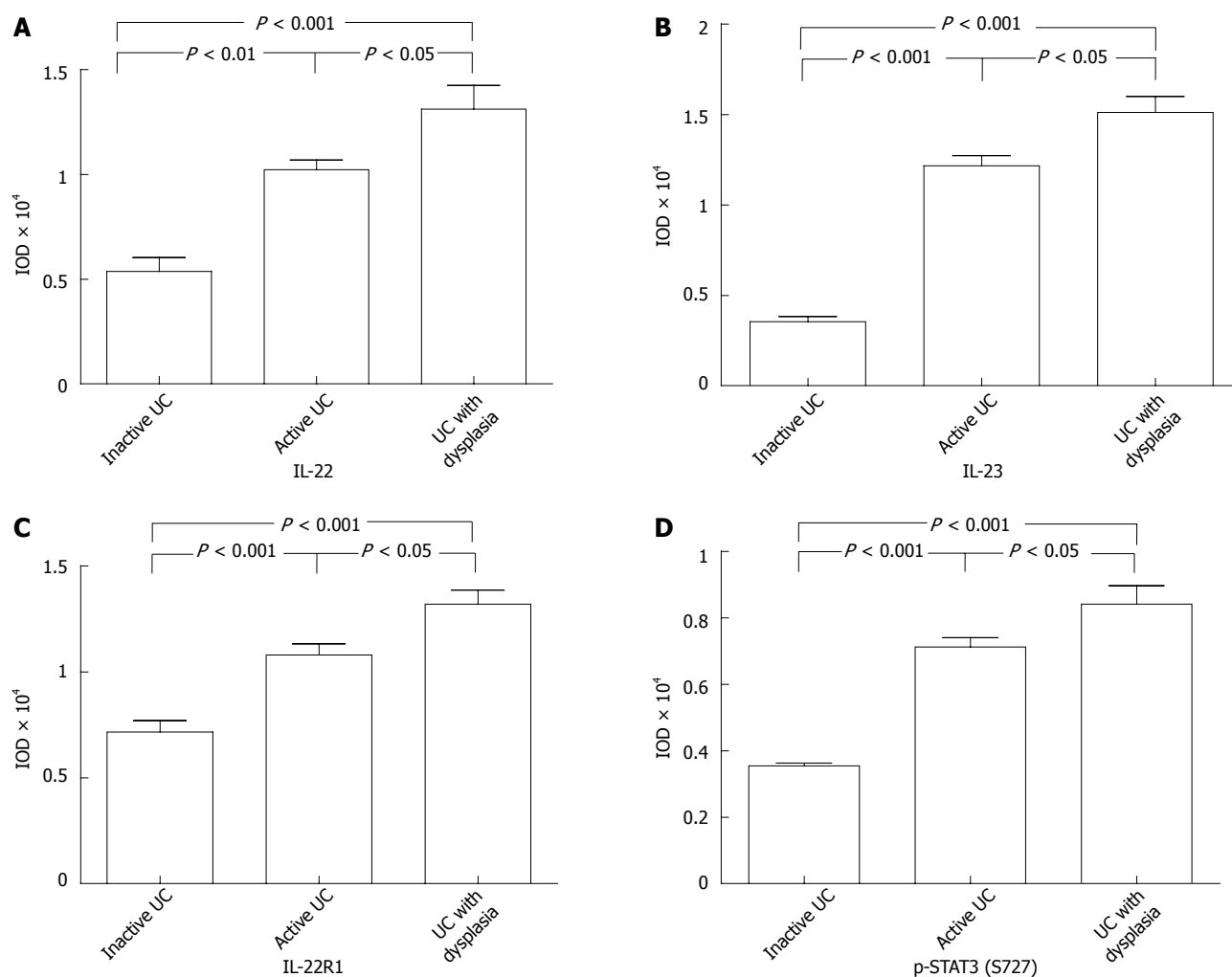


Figure 6 Statistical evaluation of interleukin-22 and its related proteins in human colonic biopsy specimens from patients with inactive ulcerative colitis, active ulcerative colitis, and ulcerative colitis with dysplasia. The average integrated optical density (IOD) for immunohistochemistry staining of interleukin (IL)-22 (A), IL-23 (B), IL-22R1 (C), and p-STAT3 (S727) (D) in colonic tissues with ulcerative colitis (UC) and UC-related carcinogenesis (UC-CRC). Significantly higher expression levels of IL-22, IL-23, IL-22R1, and p-STAT3 were observed in the dysplasia group, as compared with the inactive and active UC groups.

mining UC severity.

STAT3 is a transcription factor that is activated by the binding of several cytokines, hormones, and growth factors to their respective receptors, including IL-22, IL-6, IL-23, and IL-1 β ^[34]. We propose that STAT3 signaling is disrupted in chronic inflammation and carcinogenesis. The expression levels of total STAT3 and p-STAT3 in patients with UC were persistently elevated, with a positive correlation to the degree of inflammation^[22]. Moreover, STAT3 is constitutively activated in a variety of human cancers, including colorectal cancer. This transcription factor is crucial in cancer cells because it regulates the transcription of genes involved in cell survival, apoptosis, and other cellular processes. Morikawa *et al.*^[34] found that p-STAT3 is significantly associated with poor prognosis in a data set of 724 colorectal cancers. Furthermore, STAT3 signaling has been reported to induce cancer-promoting inflammation and to inhibit antitumor immunity^[35,36]. IL-6, a main activator of STAT3, has been proven to be important for promoting UC and UC-CRC^[37]. IL-22, another inflammatory factor that

predominantly activates STAT3, has been verified in the chronic hepatitis and hepatocellular carcinoma (HCC) microenvironment; it induces tumor growth, inhibits apoptosis, and promotes metastasis *via* STAT3 activation^[38]. Consistent with other studies on chronic hepatitis and HCC, our results demonstrated that the expression levels of IL-23, IL-22, IL-22R1, and STAT3 are consistently highly expressed in human UC tissues. IL-22 and p-STAT3, in particular, are more constitutively upregulated in the chronic colitis than in the controls. During the chronic phase of the DSS-induced mice colitis model, IL-22, IL-22R1, and IL-23 were highly expressed over time. Likewise, p-STAT3 and its downstream Bcl-2, cyclin D1, and survivin genes were remarkably upregulated. Furthermore, the expression of IL-22, p-STAT3, IL-23, and IL-22R1 were significantly elevated in human UC tissues with dysplasia, as compared with inactive and active UC tissues. Our results showed that the expression levels of IL-22 and IL-22R1 were more highly elevated in the acute colitis phase, which is in accordance with earlier studies^[27,28]. We propose that IL-22 may ameliorate

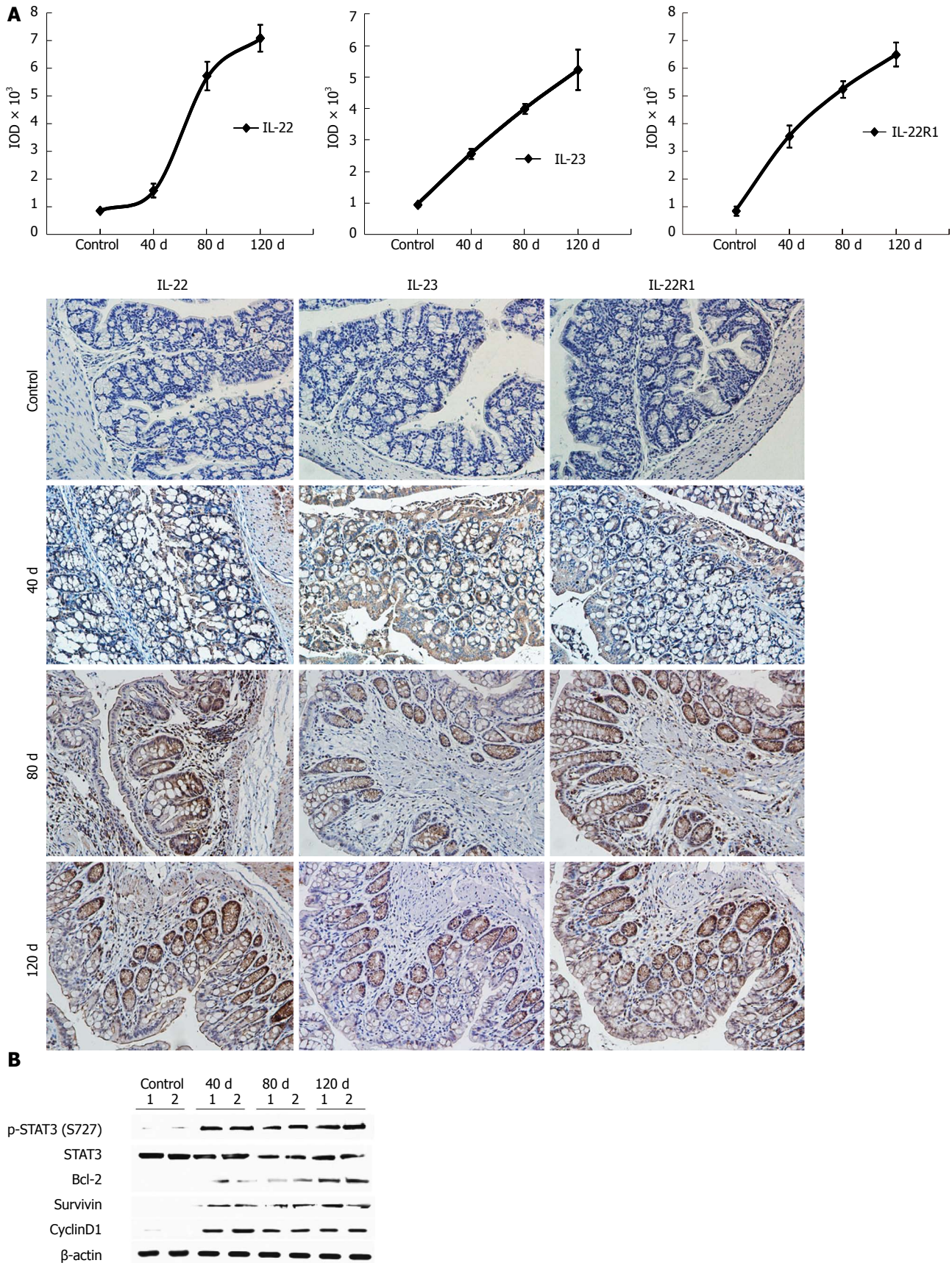


Figure 7 Expression of interleukin-22 and its related proteins in the mouse chronic colitis model. A: The average integrated optical density (IOD) for the immunohistochemistry staining of interleukin (IL)-22, IL-23, and IL-22R1 in mouse ulcerative colitis and normal tissues at different time points: days 40, 80, and 120. The expression of IL-22, IL-22R1, and IL-23 gradually increased with time; B: Western blotting detection of p-STAT3 (S727), total STAT3, Bcl-2, survivin, and cyclin D1 after DSS administration. Expression levels were all normalized to β -actin. All genes showed sustained expression over time.

intestinal inflammation by enhancing mucus production and goblet cell replacement in the early phase of inflammatory response. However, the persistent expression of IL-22 during the chronic phase of UC can strongly activate STAT3 phosphorylation in IECs, which is associated with the progression of human UC and UC-CRC^[37] by upregulating genes for cell proliferation, anti-apoptosis, and survival.

In conclusion, our study provides clinical evidence that the IL-22/STAT3 signaling pathway is related to UC and UC-CRC. Moreover, IL-22 can be used as a biomarker for determining the severity of UC and as an interesting therapeutic target in active UC and UC-CRC.

COMMENTS

Background

It has been previously reported that interleukin (IL)-22, one of the cytokines secreted by Th17 cells, promotes a protective and inflammatory effect in inflammatory bowel disease (IBD) through STAT3 signaling activation.

Research frontiers

The IL-22/STAT3 signaling pathway plays an important role in several autoimmune diseases, such as psoriasis, IBD, and so on. When activated by IL-22, STAT3 can aggravate colitis by promoting the expression of inflammatory factors such as IL-8, interferon- γ , and matrix metalloproteinases. However, some studies have found that the IL-22 induced phosphorylation of STAT3 is a defense mechanism that enhances mucus production and goblet cell replacement in mouse models of acute colitis and wound-healing. Thus, the role of the IL-22/STAT3 signaling pathway in ulcerative colitis (UC) remains unclear.

Innovations and breakthroughs

IL-22 may ameliorate intestinal inflammation by enhancing mucus production and goblet cell replacement during the early phase of the inflammatory response. However, the persistent expression of IL-22 in chronic phase of UC can strongly activate the phosphorylation of STAT3 in intestinal epithelial cells. p-STAT3 is associated with the progression of human UC and UC-related carcinogenesis (UC-CRC) because it upregulates the genes for cell proliferation, anti-apoptosis, and survival.

Peer review

The authors report about the expression of IL-22, IL-22R1, IL-23, and STAT3 in biopsies of human UC and UC-related carcinogenesis. The study is well performed; it gives an overview on the expression of the before-mentioned factors in active and chronic UC and correlates IL-22 expression with disease severity.

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