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BASIC RESEARCH

Aberrant activation of nuclear factor of activated T cell 2 in lamina propria mononuclear cells in ulcerative colitis

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Abstract

AIM: To investigate the role of nuclear factor of activated T cell 2 (NFAT2), the major NFAT protein in peripheral T cells, in sustained T cell activation and intractable inflammation in human ulcerative colitis (UC).

METHODS: We used two-dimensional gel-electrophoresis, immunohistochemistry, double immunohistochemical staining, and confocal microscopy to inspect the expression of NFAT2 in 107, 15, 48 and 5 cases of UC, Crohn's disease (CD), non-specific colitis, and 5 healthy individuals, respectively.

RESULTS: Up-regulation with profound nucleo-translocation/activation of NFAT2 of lamina propria mononuclear cells (LPMC) of colonic mucosa was found specifically in the affected colonic mucosa from patients with UC, as compared to CD or NC ($P < 0.001$, Kruskal-Wallis test). Nucleo-translocation/activation of NFAT2 primarily occurred in CD8+T, but was less prominent in CD4+ T cells or CD20+B cells. It was strongly associated with the disease activity, including endoscopic stage ($\tau = 0.2145$, $P = 0.0281$) and histologic grade ($\tau = 0.4167$, $P < 0.001$).

CONCLUSION: We disclose for the first time the nucleo-translocation/activatin of NFAT2 in lamina propria mononuclear cells in ulcerative colitis. Activation of NFAT2 was specific for ulcerative colitis and highly associated with disease activity. Since activation of NFAT2 is implicated in an auto-regulatory positive feedback loop of sustained T-cell activation and NFAT proteins play key roles in the calcium/calcineurin signaling pathways, our results not only provide new insights into the mechanism for sustained intractable inflammation, but also suggest the calcium-calcineurin/NFAT pathway as a new therapeutic target for ulcerative colitis.

Key words: Nuclear factor of activated T cells; Ulcerative colitis; Inflammatory bowel disease; Nuclear factor of activated T cells c1; Nuclear factor of activated T cells 2

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INTRODUCTION

Inflammatory bowel disease (IBD), which includes the two components of Crohn's disease (CD) and ulcerative colitis (UC), is a chronic, relapsing and debilitating idiopathic inflammation of the gastrointestinal tract. UC is characterized by crypt abscesses and ulceration of the colon, which rarely extend beyond the muscularis layer. In comparison, CD is characterized by granulomatous inflammation involving the whole layer of bowel wall and, by its extension, any portion of the gastrointestinal tract. Although the exact etiologies remain uncertain, there is compelling evidence suggesting the implication of environmental risk factors[1], including commensal bacteria[2,3], genetic predisposition[4-9], and disturbance of the immune reaction[10-12].

In both UC and CD, the inflamed tissue is heavily infiltrated with inflammatory cells, mainly T lymphocytes[11,13,14]. These cells are thought to be activated and secrete large amount of cytokines, which in turn play a primary role in the pathogenesis of the diseases[15]. In addition, based on

studies of human tissue and animal models, it has been shown that UC and CD have distinct profiles of cytokine production. UC predominantly presents with type 2 helper T cell (TH2) cytokine profiles, such as IL-4, IL-5, and IL-13, while in DC, there is primarily a secretion of type 1 helper T cell (TH1) cytokines, including IL-12, IL-23, IFN-γ and TNF-α[12,15-20]. Recently, de-regulation of innate immunity, including defects in the mucosal barrier and in innate effecter cells such as neutrophils, monocytes, and dendritic cells, has been proposed to initiate early events and perpetuate the inflammatory state in CD[21]. However, the underlying mechanisms that initiate and promote the diverse immune perturbation in IBD remain to be determined. Moreover, elucidating the mechanisms that lead to aberrant immune activation of the colonic mucosa will contribute not only to understanding disease pathogenesis but also to the development of new therapies[22-29].

Recently, we have used proteomic approaches to identify 19 differentially expressed proteins in the colonic mucosa of UC patients[30]. Of these, up-expression of the nuclear factor of activated T cells c1 (NFATc1 or NFAT2) is of particular interest, since NFAT proteins play pivotal roles in the development of the cardiovascular system and the regulation of immune function. Herein, we report results demonstrating nuclear translocation and activation of NFAT2 in infiltrating lymphocytes of UC diseased colonic mucosa.

MATERIALS AND METHODS

Patients and tissue processing

Colonic tissue sections were obtained from 107 cases of UC, 15 cases of CD, 48 cases of non-specific colitis, and 5 cases of normal controls. All UC patients, CD patients and non-specific colitis patients who underwent colonic endoscopic biopsy or surgical resection from April 1984 to September 2004 at our hospital were retrospectively included in the study in accordance with the clinical and pathologic diagnosis of UC, CD, or excluding the diagnosis of UC or CD, respectively. Those who had a tentative diagnosis of UC or CD, with a course of clinical symptoms less than three months, were excluded in this study. Seventy out of the 107 UC patients who had been regularly followed-up or were deceased by the end of the follow-up period (October, 2006) were included in the association studies between NFAT2 activation and clinical presentations. Disease activity was assayed on the basis of endoscopic observation of the mucosal pattern and histological grading[31,32].

For proteomic assays using two-dimensional gel-electrophoresis, colonic biopsy samples were taken from the inflamed mucosa in four UC patients (all males; age range 25-42 years, average age 33 years), 3 cases of non-specific, infectious colitis (males, aged 21, 28, and 43 years), and 5 individuals without obvious colonic disease (all males; age range: 24-44 years, average age 33 years). The duration of the disease for the four UC patients was 6, 4, 6, and 10 years, respectively. No patient received immunosuppressive or steroid treatment for at least three weeks before the beginning of the study. All biopsy samples were collected from the distal portion of the sigmoid colon (15-25 cm from the anal verge) and were then cut into two parts. One was immediately frozen at -80℃ for subsequent proteomic assays, while the other was fixed in fresh 4% paraformaldehyde and embedded in paraffin for histologic and immunologic staining. Diagnosis of UC was made in accordance with standard criteria. The institutional ethics committee approved all of the protocols and all enrolled patients gave their informed consent, except those whose tissue samples had been collected and stored in the tissue bank of Chang Gung Memorial Hospital before 2000.

Two-dimensional gel electrophoresis and analysis

The methods used for two-dimensional gel electrophoresis have been described previously[1]. 29 In brief, frozen tissue samples (200 mg) were homogenized in 2 mL homogenization buffer (50 mmol/L Tris-HCl, pH 7.2) containing a protease inhibitor cocktail (1 mmol/L 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, 0.8 mmol/L aprotinin, 21 mmol/L leupeptin, 36 mmol/L bestatin, 15 mmol/L pepstatin A, 14 mmol/L E-64) using a homogenizer (IKA Labotechnik, Staufen, Germany) at 25 000 r/min. Samples containing 120 μg protein were subjected in isoelectric focusing using the IPG strips in Protean IEF Cell (BioRad) in accordance with the manufacturer's instructions. Separation in the second dimension of 12.5% polyacrylamide gel slabs was carried out using Protean Ⅱ electrophoresis equipment (BioRad).

The gels were initially fixed in 10% methanol and 7% acetic acid, and then stained for 3 h in a commercially available SYPRO Ruby buffer (Molecular Probes, Eugene, OR). Protein patterns in the gels were recorded as digitalized images using a high-resolution scanner (GS-710 Calibrated Imaging Densitometer, Bio-Rad). Gel image matching was done with Progenesis software (Progenesis Discovery, Nonlinear Dynamics, Durham, NC).

Protein identification by mass spectrometry

Protein spots of interest were manually excised from the sypro Ruby stained 2D gels, de-stained using 50 mmol/L NH_4HCO_3 in 50% acetonitrile and dried. The protein was digested by overnight incubation at 37℃ with trypsin at 5 ng/mL in 50 mmol/L NH_4HCO_3, pH 7.8, followed by extraction in 1 volume 0.1% TFA and being eluted in 1.5 μL matrix (5 mg α-cyano 4-hydroxycinnamic acid/mL in 50% acetonitrile/0.1% TFA) for MALDI-TOF MS and MS/MS analysis. Both MS and MS/MS spectra were searched against the NCBI database, using the mascot software from matrix science (www.matrixscience.com) to identify the proteins.

Immuno-histochemical, double immunofluorescence and confocal microscopic studies

For immuno-histochemistical studies, after being de-waxed and rehydrated, the tissue sections were incubated with mouse anti-human NFAT2 as primary antibodies (diluted 1:500 in PBS; Abcam, Cambridgeshire, UK) for 2 h at room temperature, followed by rinsing with PBS and incubation by an HRP-conjugated rabbit anti-mouse IgG, or Rhodamin-conjugated anti-mouse IgG

as the second antibody. HRP activity was detected using diaminobenzidine tetrahydrochloride (DAB) as substrate for 3 min in accordance with the manufacturer's instructions (BioGenex, San Ramon, CA 94583 USA).

To determine the relationship between the expression of NFAT2 and the cell types of LPMCs, we performed double immunofluorescence staining for NFAT2 and CD3 (BD, Franklin Lakes, NJ USA), CD4 (DAKO, Glostrup, Denmark), CD8 (Hytest, Turku Finland), and CD20 (DAKO, Glostrup, Denmark) on the same sections using Envision Doublestain System (DAKO, Glostrup, Denmark). Deparaffinization and microwave antigen retrieval were performed as described above. After quenching the endogenous peroxidase activity with peroxidase blocking reagent (DAKO, Glostrup, Denmark), tissue sections were incubated with mouse monoclonal anti-CD antibodies for one hour at room temperature, followed by rinsing with washing buffer and were incubated with HRP-conjugated anti-mouse antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) for 30 min at room temperature with detection of peroxidase activity. Then the tissue sections were subjected to the second staining for NFAT2 with the sequential steps of quenching the endogenous phosphatase, incubation with anti-NFAT2 antibodies, incubation with anti-goat IgG antibodies and detection of the phosphatase activity in accordance with the manufacturer's instructions (Envision Doublestain System, DAKO, Glostrup, Denmark).

For confocal microscopy, tissue sections were de-waxed using xylene twice and rehydrated with PBS followed by blocking with goat serum (BioGenex, San Ramon, CA 94583 USA) for 10 min at room temperature. The tissue sections were then incubated for 2 h with the specific antibody against NFAT2 (1:50, ab25916, Abcam, plc), rinsed extensively three times with PBS, incubated with secondary antibody (1:200 Jackson ImmunoResearch) for 30 min at room temperature in the dark, and then counterstained with 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI, Sigma-Aldrich). The sections were rinsed successively with PBS, distilled water, and ethanol, and mounted with a drop of Mowiol on a micro slide. Confocal microscopy was performed using a laser scanning spectral confocal microscope (Leica PCS ST2, Leica Microsystems, Wetzlar, Germany).

Statistical analysis

The degree of nucleotranslocation of NFAT2 was classified into five grades (Ⅰ: 0%; Ⅱ: < 10%; Ⅲ: 10%-50%; Ⅳ: 50%-90%; Ⅴ: > 90% of LPMC with nucleotranslocation of NFAT2) or low (< 50%) or high (> 50%). Comparing the degree of nucleo-translocation of NFAT2 among UC, CD and non-specific colitis patients, we used Kruskal-Wallis test (for UC, CD and NC three groups) or Mann-Whitney test (for any two unpaired groups). Associating the onset age with the degree of NFAT nucleo-translocation (< 50% *vs* > 50%) in UC patients, we used an unpaired two-sample *t* test. Examining the association of gender, sex, and disease duration before sampling with the degree of NFAT nucleo-translocation (< 50% *vs* > 50%), we used a corrected χ^2 test. To test association of the degree of nucleo-translocation of NFAT2 (< 50% *vs* > 50%) to the clinical outcome of UC patients, we used a two-side tailed Fisher's exact test. Correlating the degree of NFAT2 nucleo-translocation (grade Ⅰ-Ⅴ) to the disease activity (endoscopic grade 1-4 and histological grade 1-4) of UC patients, we used Kendall's -rank correlation coefficient.

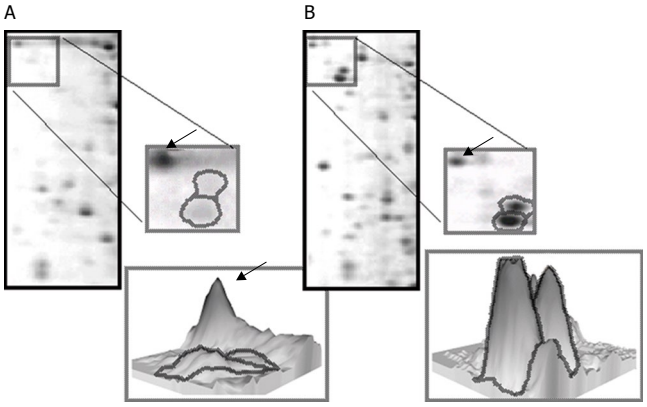

Figure 1 Two-dimensional gel electrophoresis identifying increased amounts of NFAT2 in UC colonic mucosa tissue. Tissue protein lysate was prepared from normal (A) and UC affected colon tissues (B) and separated using two-dimensional gel electrophoresis (2-DE). Spot identification and matching across the gels and determination of the relative amount for each corresponding protein spot were conducted using the software, Progenesis workstation (non-linear). Proteins were identified using mass spectrometry as previously described[30]. The representative results of 2-DE are shown. The inserts are the close views along with the 3-dimensional pictures of NFAT2 and the reference protein spot on the 2-DE. Arrows indicate the reference protein spots across gels. The grey lines outline the NFAT2 spots.

RESULTS

Up-expression of NFAT2 in the proteomes of UC colonic mucosa

To identify the de-regulated proteins in colonic mucosa of UC, two-dimensional gel electrophoresis was used to compare the colon-mucosa proteomes between four UC patients and three healthy controls. Of interest is the up-expression of NFAT2 in UC colonic mucosa (Figure 1), because of its potential role in regulating immune function.

Nucleo-translocation/activation of NFAT2 in the mucosa infiltrating lymphocytes in UC colonic tissues

To validate the up-expression of NFAT2 in UC diseased colonic mucosa, immuno-histochemistry was used to examine the relative number of cells specifically expressing NFAT2 in colonic mucosal tissue sections obtained from healthy controls and UC and CD patients. As shown in Figure 2, NFAT2 was detected in LPMCs obtained from the UC (Figure 2 D-F) and CD patients (Figure 2 G-I), as well as from the healthy controls (Figure 2 A-C), indicating that the up-expression of NFAT2 in UC was primarily due to the increase in the number of mucosa infiltrating lymphocytes as well as the up-regulation of NFAT2 in each infiltrating lymphocyte.

However, high magnification view showed differential distribution of NFAT2 inside the mucosa infiltrating lymphocytes among UC, CD, and controls. NFAT2 was restricted in the cytoplasm of lymphocytes in normal

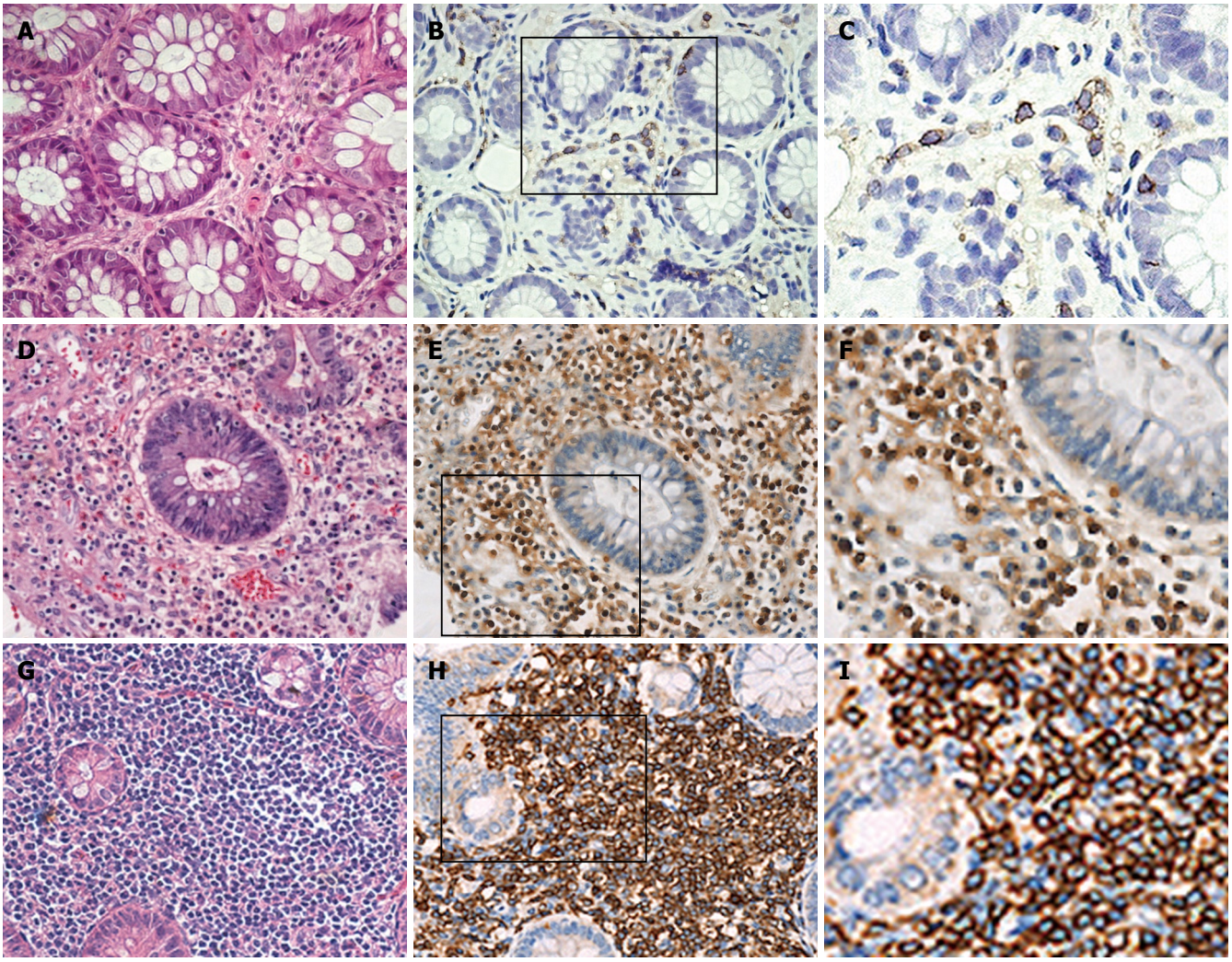

Figure 2 Immunohistochemical analysis of the expression of NFAT2 in colon mucosa tissues. **A-C** are derived from a case of normal control, **D-F** from a case of UC, and **G-I** from a case of CD. **A**, **D** ,**G** are the results of H&E stain in 100 × magnification. **B**, **E**, **H** are the results of immunohistochemistry for NFAT2 counter-staining with hematoxylin for nuclei, 100 × magnification. **C**, **F**, **I** are the close view of **B**, **E**, **H** respectively. Of note, NFAT2 was exclusively located in cytoplasm of LMPCs of normal colon mucosa as well as LMPCs of CD affected colonic mucosa, whereas NFAT2 was primarily located in the nuclei of LMPCs of the UC affected colonic mucosa.

colonic mucosa (Figure 2B and C), while it was primarily located in the nuclei of infiltrating lymphocytes in UC colonic mucosa (Figure 2E and F). NFAT2 was also restricted in the cytoplasm of infiltrating lymphocytes in CD colon tissue (Figure 2H and I), although there was a heavy infiltration of lymphocytes (Figure 2G-I). Nucleo-translocation of NFAT2 within the infiltrating lymphocytes in UC colonic mucosa, but not in CD affected or normal colonic mucosa, was further validated by confocal microscopy, in which the subcellular distribution of NFATA2 was co-localized with chromosomal DNA within the nuclei of LPMCs in UC colon mucosa (Figure 3).

The cell-types of LMPC with NFAT2 nucleo-translocation/activation in the UC affected colonic mucosa were determined by double-staining using monoclonal antibodies against either CD3, CD4, or CD20, with those against NFAT2. The studies revealed that that nucleo-translocation of NFAT2 primarily occurred in CD8+ T cells and less prominent in CD4+ T cells (data not shown). NTAF2 was also expressed in some of the CD20+ B cells and translocated into nuclei, but mainly localized in the peripheral region of the nuclei (data not shown).

Nucleo-translocation/activation of NFAT2 specifically in UC

To further examine whether nucleotranslocation of NFAT2 of LPMCs in UC, we compared the degree of nucleotranslocatio of NFAT2 in LMPCs in UC, DC and non-specific colitis. Diseased colonic tissue sections were obtained from 107 UC cases, 15 CD cases, and 48 cases of non-specific colitis. As shown in Figure 4 and summarized in Table 1, 76%, 26.7% and 8.3% of UC, CD and NC patients had high degree (Grade Ⅳ and Ⅴ) of nuclear translocation of NFAT2, whereas 12%, 60% and 83% of UC, CD and NC patients had low degree (Grade Ⅰ and Ⅱ) of nucleotranslocation of NFAT. Obviously, nuclear translocation of NFAT2 within LPMCs was specific for ulcerative colitis ($P < 0.001$ for all three groups, *via* Kruskal-Wallis statistic test; $P < 0.001$ for UC *vs* CD; $P < 0.001$ for UC *vs* NC; $P = 0.023$ for CD *vs* NC, *via* Mann-Whitney test).

Nucleo-translocation/activation of NFAT2 and endoscopy, histologic grading, and clinical outcome

The degree of nucleotranslocation of NFAT2 in LPMCs was correlated to the clinical presentations of the cases

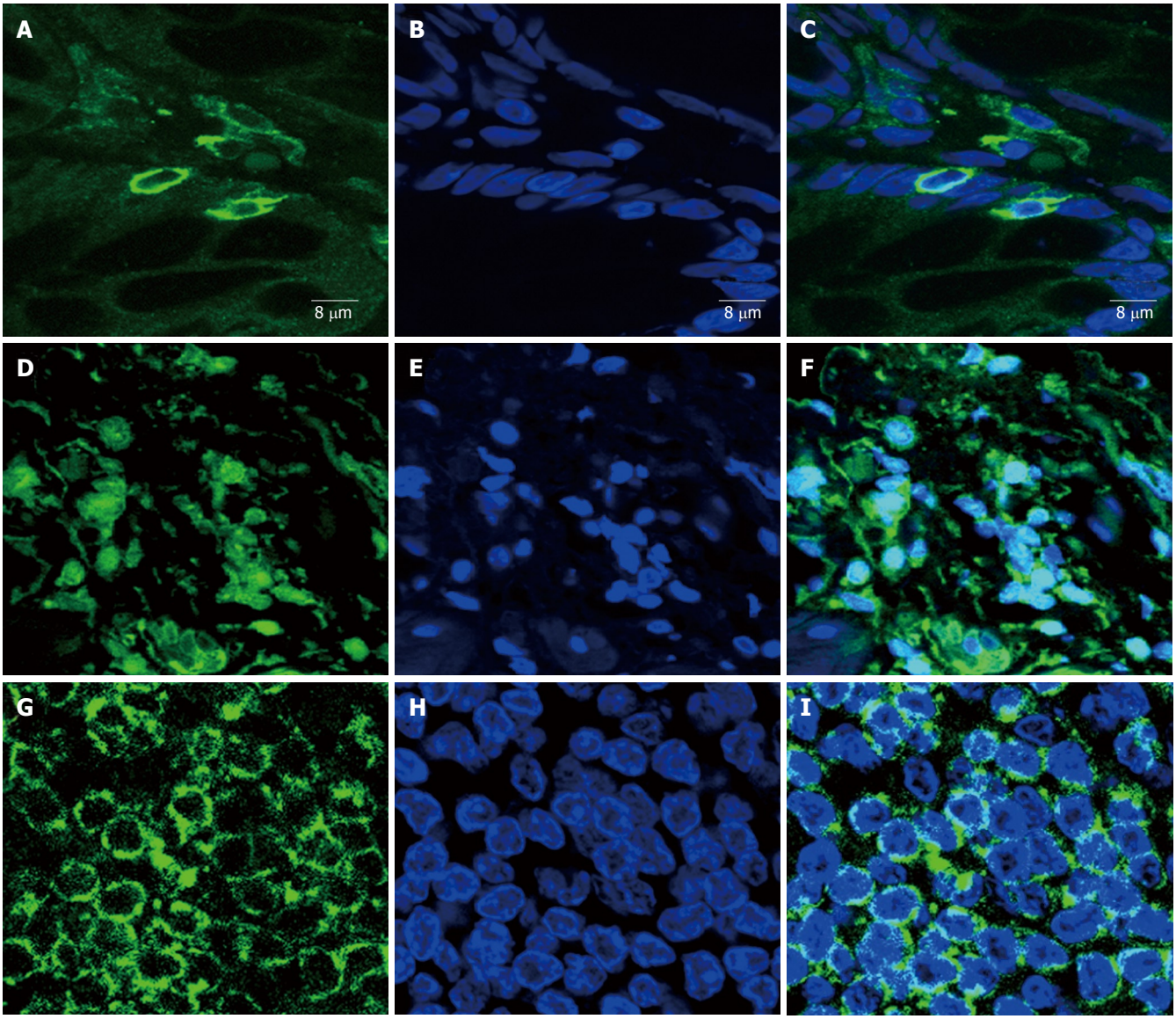

Figure 3 Confocal microscopy demonstrating the subcellular distribution of NFAT2 in LMPCs of colon mucosa. **A**-**C** are derived from normal colon mucosa, while **D**-**F** from UC affected mucosa, **G**-**I** from CD affected mucosa. **A**, **D**, **G** represent the detection for NFAT2; **B**, **E**, **H**: The nuclei using DAPI; **C**, **F** ,**I** : the merged images for **A**, **B**, **D**, **E**, **G** and **H**, respectively. Of interest, co-localization of NFAT2 with nuclei is noted in the colonic mucosa of UC (**F**), but not in CD (**I**), though there is a heavy infiltration of LMPCs in the CD affected colonic mucosa.

of UC, including gender, age at onset, disease duration before sampling, mucosal pattern (endoscopy grading), histologic grading, and outcome (Table 2). The degree of nucleo-translocation/activation of NFAT2 was not related to patients' gender, onset age, and disease duration. It was, however, highly associated with endoscopic grade (correlation coefficient, $\tau = 0.2145$, $P = 0.0281$ *via* Kendall's-rank correlation coefficient), and the histologic grade (correlation coefficient, $\tau = 0.4167$, $P = 0.000$ *via* Kendall's-rank correlation coefficient) and vice versa. There was no significant association between the degree of nucleotranslocation of NFAT2 and long-term clinical outcome of UC including requiring surgical intervention ($P = 0.164$ *via* Fisher's exact test) and survival ($P = 0.081$, *via* Fisher's exact test) (Table 2).

On the other hand, the outcome of long-term follow-up was strongly associated with disease duration before sampling ($P = 0.000$ *via* Kendall's-rank correlation

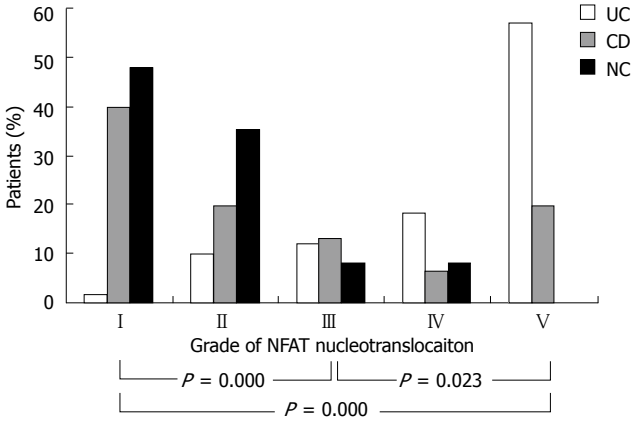

Figure 4 Nucleo-translocation of NFAT2 in UC, CD, and non-specific colitis. Nucleo-translocation of NFAT2 was assayed using immuno-histochemical staining and the tissue sections were obtained from a total of 107 cases of UC, 15 cases of CD and 3 cases of non-specific colitis (NC). Overall *P* value < 0.001 (determined *via* Kruskal-Wallis statistic).

Table 1 Comparison of the degree of NFAT2 nucleo-translocation in LPMCs in patients of UC, CD and non-specific colitis

Grades of NFAT2 nucleo-translocation in LPMCs (%)	Ulcerative colitis (cases) (%)[1]	Crohn's disease (cases) (%)[1]	Non-specific colitis (cases) (%)[1]
Ⅰ (0)	2 (01.9)	6 (40.0)	23 (47.9)
Ⅱ (< 10)	11 (10.3)	3 (20.0)	17 (35.4)
Ⅲ (10-50)	13 (12.1)	2 (13.3)	4 (8.3)
Ⅳ (50-90)	20 (18.7)	1 (06.7)	4 (8.3)
Ⅴ (> 90)	61 (57.0)	3 (20.0)	0 (0.00)
Total cases	107	15	48

[1]P = 0.000 amongst UC, CD and NC groups, *via* Kruskal-Wallis statistics; P = 0.000 for UC *vs* CD, *via* Mann-Whitney's test; P = 0.000 for UC *vs* NC, *via* Mann-Whitney's test; P = 0.023 for CD *vs* NC, *via* Mann-Whitney's test.

Table 2 Correlation of NFAT2 nucleotranslocation to clinical presentations

	P value	Statistic methods
Degree of NFAT2 nucleotranslocation[1]		
Onset age (yr, mean ± SD)[1]	0.9484	*t*-test
Gender (M *vs* F) [1]	0.651	χ^2 test
Disease duration (< 3 m *vs* > 3 m) [1]	0.507	χ^2 test
Outcome (survived *vs* non-survived[3])[1]	0.081	Fisher's exact test
Outcome (colectomy *vs* non-colectomy)[1]	0.164	Fisher's exact test
Degree of NFAT2 nucleotranslocation[2]		
Endoscopic grade (1-4)[2]	0.0281	Kendall's-rank correlation coefficient τ = 0.2145
Histological grade (1-4)[2]	0	Kendall's-rank correlation coefficient τ = 0.4167

[1]The degree of NFAT2 nucleo-translocation is divided into < 50% *vs* > 50% of LPMCs. [2]The degree of NFAT2 nucleo-translocation is categorized into 0%, < 10%, 10%-50%, 50%-90%, > 90% of LPMCs. [3]Non-survived: Died of complications of UC.

coefficient), endoscopy grade (P = 0.000 *via* Kendall's-rank correlation coefficient) and histologic grade (P = 0.031 Kendall's-rank correlation coefficient), but not associated with the degree of nucleo-translocation/activation of NFAT2 (P = 0.185 *via* Kendall's-rank correlation coefficient), age (P = 0.406), or sex (P = 0.369).

DISCUSSION

Though many studies and reports have addressed the role of cytokines in the development of IBD, there is still a great gap between the increase of the putative initiating cytokines, such as IL-4 and IFN-γ (around 3 fold), and the downstream profound cascade of pro-inflammatory cytokines that include TNF-α, IL-1β and IL-6 (around 10-20 folds), which are directly implicated in the inflammatory process[10-12,33,34]. Indeed, the mechanisms that multiply downstream signaling and cause intractable inflammatory events remains completely unclear.

We report here the nucleo-translocation/activation of NFAT2 of lamina propria mononuclear cells (LPMCs) in UC affected colonic mucosa. The nucleo-translocation/activation of NFAT2 of LPMCs is specific for UC because it is relatively rare in CD and is rarely found in non-specific colitis. Of interest, it has been shown that the activation of NFAT2 primarily leads to the commitment of Th2 differentiation[35-37]. Our findings of the nucleo-translocation/activation of NFAT2 specifically in UC is consistent with the general notion that UC is primarily driven by a Th2-like immune activation that is different from the major roles of Th1 immune activation in CD[10-14]. In addition, we show that the degree of nucleo-translocation/activation of NFAT2 of LPMC has been strongly associated with disease activity, such as clinical and histological grading, indicating its direct implication in the pathogenesis of UC. Our findings are reminiscent of that reported by Neurath *et al*, in which activation of T-bet, a transcription factor directing Th1 cell development and regulating T cell function, in the LPMCs was specifically found in CD patients but not in UC patients[38]. In addition, over-expression of T-bet was found to be essential and sufficient to promote Th1-mediated colitis in an experimental mouse model of CD[38].

NFAT proteins, which are originally identified in T cells as inducers of cytokine gene expression, play a variety of biological roles in the differentiation of many cell types, including effector T cells, osteoclasts, and muscle cells[36,37,39-41]. The NFAT family contains five transcription factors, four of which (NFAT1-4) are regulated by the calcium/calcineurin signaling pathways that are essential for the regulation of lymphocyte function. The only non-calcium-regulated NFAT protein, NFAT5, is expressed in response to osmotic stress by almost all cells[40,42].

Activation of NFAT1-4 proteins is tightly regulated by calcium-dependent phosphatase calcineurin. Calcineurin signaling was first defined in T lymphocytes as a regulator of nucleo-translocation and activation of NFAT proteins. In resting cells, NFAT proteins reside in the cytoplasm and are heavily phosphorylated. Upon engagement of cell surface receptors, the membrane component phosphatidylinositiol-4,5-bisphosphate is converted to inositol-1,4,5-trisphosphate (IP3) and diacylglycerol. IP3 sequentially induces the calcium release from the intracellular store and triggers the opening of calcium-release-activated calcium channels in cytoplasm membrane, thereby maintaining the increased levels of intracellular calcium. This, in turn, forms a complex with calmodulin that activates the Ser/Thr-phosphatase activity of calcineurin so as to de-phosphorylate NFAT proteins resulting in nucleo-translocation of NFAT proteins and the induction of NFAT-dependent gene transcription[40,43,44].

NFAT1 and NFAT2 are the major NFAT proteins in peripheral T cells; they overlap in function but differ remarkably in mode of expression. NFAT1 is constitutively synthesized in T cells, whereas NFAT2, the most prominent NFAT proteins in peripheral T cells, is expressed upon following T-cell receptor and co-receptor stimulation and maintained by an auto-regulation mechanism[40,45]. Activation of NFAT proteins, together with other transcription factors (such as T-bet, STAT1, 4, 6, GATA3, and FOXP3), determines the TH1/TH2/Treg lineage choice, particularly the development of TH2 immune response that is dependent on the nature of the stimulus and the signals that are received

from specific cytokines[34,40,45,46]. Upon the engagement of TCR, for example, NFAT2 cooperates with MAT to induce the expression of IL-4, IL-5 and TH2 lineage specific transcription factors (such as NFAT2, STAT6 and GATA3) to commit to TH2 cell differentiation[36,37,40,47-49].

Of interest, there are two NFAT binding sites in each of the two promoter regions of the NFAT2 gene. Mutations abolishing NFAT binding result in a profound decrease in the transcription of NFAT2. Obviously, there is a positive auto-regulation of NFAT2 expression in TCR activated T cells[43,50,51]. Taken together, nucleo-translocation/activation of NFAT2 inflames an auto-regulatory positive feedback loop in amplifying downstream pro-inflammatory signaling. Our findings of specific nucleo-translocation/activation of NFAT2 in LPMCs in UC might imply the answer to the long term enigma of the mechanisms leading to the ultimate cascade of intractable inflammatory process of UC[52].

The strong association of aberrant nucleotranslocation/activation of NFAT2 in LPMCs with the disease activity of UC further supports our hypothesis of direct implication of the calcium-calcineurin/NFAT pathways in the pathogenesis of UC. Blocking the positive-feedback loops initiated by the activation of NFAT2 would not only achieve an instant anti-inflammatory effect but might also lead to a long term remission of the disease. The calcium-calcineurin/NFAT pathways can therefore be the therapeutic targets for UC treatment. Indeed, targeting the calcium-calcineurin/NFAT pathways in the treatment of other chronic immune-mediated and intractable auto-immune diseases has been well documented[53]. For the treatment of UC, cyclosporin A, a specific inhibitor of calcineurin activity, has also been reported to be a successful treatment in cases with severe or steroids intractable UC[22,54-59]. Cyclosporin A has also been suggested as the consensus treatment for patients with severe UC in whom standard therapy has failed, or for those who are candidates for colectomy[60]. Nevertheless, the clinical use of the potent immunosuppressants, such as cyclosporin A (CsA) and tacrolimus (FK506), the fungal metabolites blocking the phosphatase activity of calcineurin, and consequently inhibiting the de-phosphorylation and nucleo-translocation of NFAT proteins, can cause severe side effects and, as such, their prolonged use in many chronic inflammatory or autoimmune diseases has been restricted. Recently, there has been a merging of peptide sequences that more specifically interfere with the interaction between calcineurin and NFAT, as well as small organic molecules that modulate NFAT function as the potential new immunosuppressive drug with improved specificity and reduced toxicity. Application of similarly newly developed drugs to the treatment of UC and some of CD will open the new era of IBD therapy in the near future[22-29,61-63].

In summary, since the activation of NFAT is implicated in an auto-regulatory positive feedback loop of sustained T-cell activation, our findings of specific nucleo-translocation/activation of NFAT2 in LPMCs of UC not only indicate the pivotal roles of activation of NFAT2 pathways in the intractable inflammatory process of UC for the first time, but also open a window for the future development of new strategies for UC therapy.

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COMMENTS

Background

We used two-dimension gel electrophoresis comparing the proteome difference between the ulcerative colitis (UC). Affected and unaffected colonic mucosa. Of the up-regulated proteins in the UC affected colonic mucosa, nuclear factor of activated T cell 2 (NFAT2)was particularly interesting, since NFAT2 is not only the major NFAT proteins in peripheral T cells but also playing key roles in regulating T cell differentiation and functions. We aimed to investigate the role of NFAT2 in the development of UC.

Research frontiers

Although T cells play pivotal roles in the pathogenesis of ulcerative colitis, the mechanisms causing sustained T cell activation and intractable inflammation remain unknown. It has been shown that activation of NFAT2 is implicated in an auto-regulatory positive feedback loop leading to a sustained T-cell activation. However, the role of NFAT in the pathogenesis of UC has never been addressed.

Innovations and breakthroughs

This is the first study addressing the strong association of nucleo-translocation/activation of NFAT2 of the lamina propria lymphocytes in the UC affected mucosa with the disease activity and severity. More interestingly, nucleo-translocation/activation of NFAT2 of the lamina propria lymphocytes in the colonic mucosa was specific for UC, since it was less prominent in Crohn's disease (CD) and rarely detected in non-specific colitis, suggesting the roles of NFAT2 activation in the pathogenesis of UC.

Applications

Because NFAT proteins are regulated by the calcium/calcineurin signaling pathways, our findings suggest direct implication of the calcium/calcineurin signaling pathways in the pathogenesis of UC. Blocking the positive-feedback loops initiated by the activation of NFAT2 would not only achieve an instant anti-inflammatory effect but might also lead to a long term remission of the disease. The calcium-calcineurin/NFAT pathways can therefore be the therapeutic targets for UC treatment.

Terminology

NFAT proteins originally identified in T cells as inducers of cytokine gene expression play a variety of biological roles in the differentiation of many cell types, including effecter T cells, osteoclasts, and muscle cells. NFAT2 is the most prominent NFAT proteins in peripheral T cells. Activation of NFAT2 determines the TH1/ TH2/Treg lineage choice, particularly the development of TH2 immune response.

Peer review

In this study, the authors demonstrated for the first time the strong association of activation of nuclear factor of activated T lymphocytes with the disease activity and severity of UC. This work adds significant information regarding the mechanisms of sustained T cell activation and intractable inflammation of UC, and opens a new window for future development of anti-UC therapy.

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