Int J Clin Exp Pathol 2013;6(5):917-925
www.ijcep.com /ISSN:1936-2625/IJCEP1303048

Original Article

Intestinal protein expression profile identifies inflammatory bowel disease and predicts relapse

Jun Shen[1,2], Yuqi Qiao[1,2], Zhihua Ran[1,2], Tianrong Wang[1,2], Jiangtao Xu[3], Jinsun Feng[3]

[1]Division of Gastroenterology and Hepatology, Shanghai Jiao-Tong University School of Medicine Renji Hospital, Shanghai Institute of Digestive Disease; [2]Key Laboratory of Gastroenterology & Hepatology, Ministry of Health (Shanghai Jiao-Tong University), Shanghai, 200127, China; [3]National Engineering Center for Biochip at Shanghai, Shanghai, 201203, China

Received March 23, 2013; Accepted April 8, 2013; Epub April 15, 2013; Published May 1, 2013

Abstract: To date, most studies have applied individual factors as indicators of disease classification and prognosis. The aim of this study is to determine whether clustering analysis of protein expression profiles in intestinal epithelia improves classification and prognosis in patients with inflammatory bowel disease (IBD). One hundred and twenty Crohn's disease (CD) patients, 117 ulcerative colitis (UC) patients and 120 cases of nonspecific colitis provided intestinal biopsy samples for tissue microarray (TMA). Both unsupervised and supervised analyses were used for evaluation of clustering and association with relapse. There was a significant concordance between cluster groups based on immunostaining data of TMA and clinical classification in distinguishing IBD from nonspecific colitis (kappa=0.498, p<0.001). CD27, CD70, CD40, TRAF3, TRAF4 and TRAF2 presented similar immunostaining features, which were different from clusters of CD154, CD80 and TRAF5. Moreover, higher expression of TRAF2 was a predictor of relapse in patients with UC (p=0.006).Thus, protein expression profiles can distinguish IBD and nonspecific colitis, and combination analysis protein expression profiles show that TRAF2 can predict relapse of UC.

Keywords: Crohn's disease, ulcerative colitis, classification, relapse

Introduction

Lower GI tract inflammation can be divided into highly heterogeneous groups of diseases, and a major differential diagnosis is inflammatory bowel disease (IBD) [1]. When patients present with symptoms suggestive of IBD, combinations of invasive and non-invasive tests can be used to help distinguish nonspecific colonic inflammation from IBD, or distinguish Crohn's disease (CD) from ulcerative colitis (UC), which are the main subforms of IBD [2]. Chronic inflammation to intestinal mucosa imparts many histologic abnormalities that may reinforce the clinical impression and narrow the differential diagnosis, which is especially important if the pathogen for the inflammation remains unclear. A correct classification of chronic inflammatory injury to ileocolonic mucosa is important for the success of both medical and surgical therapeutic strategies [3]. Despite the advent of new molecular technologies for the examination of serum proteins and genetic sequences, the diagnosis and evaluation of CD and UC based on endoscopic and histologic criteria remain unchanged [4]. More importantly, it is known that failure to achieve mucosal healing with therapy is associated with worse disease course [5]. However, the precise diagnosis of CD or UC cannot always be established with the available diagnostic tools because of overlapping features of CD and UC [6]. Although various histological patterns reflect severity and duration of IBD, few samples have specific diagnostic features [7]. Therefore, the correlation with endoscopic and clinical information is essential to receive a specific diagnosis and a fair evaluation of IBD.

Recently, there has been an increase in interests to discover new biomarkers of IBD to predict future patterns of disease and to help diagnosis, treatment, and prognosis. Most patients will probably alternate between remission and

Table 1. Characteristics of patients with inflammatory bowel disease

	CD (*n*=120)	UC (*n*=117)	NC (*n*=120)
Age at onset (yrs)	33.20 (30.64-35.76)	40.84 (37.98-43.69)***	34.25 (31.60-36.90)
BMI (kg/m^2)	19.25 (18.98-19.52)***	19.92 (19.61-20.22)***	22.22 (21.82-22.63)
Gender (Female/Male)	48/72	51/66	46/74
Relapse (*n*)†	78	65	
Extent			
Ileitis	7		
Ileocolitis	57		
Colitis	56		
Proctosigmoiditis		31	
Left sided colitis		51	
Pancolitis		35	
Therapy			
5-ASA/ SASP	101	108	
Glucocorticoid	46	57	
Azathioprine	19	9	
Infliximab	12	6	

CD, Crohn's disease; UC, ulcerative colitis; NC, nonspecific colitis; BMI, body mass index; 5-ASA, 5-aminosalicylic acid; SASP, salicylazosulfapyridine. †End point is designated as one year after remission; ***Significantly different from nonspecific colitis, $p<0.001$.

relapse, with 10% having a relapse-free course after 10 years, and few having a continuously active course [8]. To optimize prognosis, it is important to identify prognostic factors that predict disease course at disease onset [9]. Several biomarkers have been studied in IBD as diagnostic aids, indicators of disease activity or severity, and to predict the risk of relapse in those patients in remission [10, 11]. However, none of these individual biomarkers has enough high sensitivity and specificity for accurate differential diagnosis among CD, UC and other nonspecific colitis. Individual factors as indicator of disease activity and prognosis are still conflicting, rigorous additional studies [12, 13]. Thus, panels of biomarkers are considered by clinicians for the management of IBD patients. One of the opportunities to identify and/or validate molecular signatures is provided by alternative high-through put approaches such as tissue microarrays (TMA) [14, 15]. Immunohistochemistry on TMA may be a practical approach both in validation studies and in routine testing.

To date, most studies only apply individual factor as indicator of disease classification and prognosis, while seldom have addressed the unsupervised and supervised analysis. The aim of present study is to determine whether clustering analysis of protein expression profiles in intestinal epithelia improves classification and prognosis in patients with IBD.

Patients and methods

Patients and samples

This study comprised 120 CD patients and 117 UC patients who underwent endoscopy between Dec 2006 and Dec 2009 at Renji Hospital, Shanghai Jiao-Tong University School of Medicine. 120 cases of nonspecific colitis were obtained from Renji Hospital between Jan 2009 and Jan 2010 after informed consent. More than 600 biopsy specimens were studied using tissue microarrays. Patients with IBD were followed for one year after inducing remission, or less if they relapsed. Inclusion criteria were: clinical remission for at least 1 month at study entry as defined by a Crohn's Disease Activity Index (CDAI) of less than 150 or Sutherland Disease Activity Index. Exclusion criteria were: pregnancy; previous small bowel resection or colostomy; use of prednisone or budesonide within 30 days of study entry; and antibiotic use at study entry. Patients receiving oral mesalamine, azathioprine or methotrexate were excluded if their medication dose had been altered within 30 days (oral mesalamine)

Table 2. Characteristics of antibodies and quick scores of tissue microarrays

Proteins	CD (quick score)	UC (quick score)	NC (quick score)	*P* value
CD27	4 (2-6)***	2 (1-4)	2 (1-4)	<0.0001
CD70	2 (1-4)*	3 (2-6)***	2 (0-3)	<0.0001
TRAF2	2 (1-4)**	6 (4-9)***	3 (3-6)	<0.0001
TRAF3	1 (0-2)**	1 (0-1)***	1 (1-2)	<0.0001
TRAF4	2 (1-6)**	3 (2-6)	3 (3-6)	0.0046
TRAF5	6 (4-8)***	3 (2-6)***	12 (6-12)	<0.0001
STAT3	2 (0-3)	2 (1-3)***	2 (0-3)	0.0002
CD40	0 (0-1)	0 (0-1)	0 (0-1)	0.0852
CD154	8 (4-12)	4 (3-8)***	8 (8-8)	<0.0001
CD80	6 (2-9)***	2 (1-6)***	8 (6-12)	<0.0001

*Significantly different from nonspecific colitis, p <0.05. **Significantly different from nonspecific colitis, p <0.01. ***Significantly different from nonspecific colitis, p <0.001. CD, Crohn's disease; UC, ulcerative colitis; NC, nonspecific colitis; Mmab, Mouse monoclonal antibody; Rpab, Rabbit polyclonal antibody; Rmab, Rabbit monoclonal antibody; data were presented as median and 25%-75% percentile.

or within 3 months (azathioprine or methotrexate) before study entry. Ethical approval for the study was obtained from the Research Ethics Committee of Renji Hospital, Shanghai Jiao-Tong University School of Medicine.

Simple Endoscopic Score for Crohn's Disease (SESCD) [16] and Baron score [17] were used for the endoscopic evaluation of CD and UC respectively. Patients were instructed to communicate with the research coordinator if they developed symptoms suggestive of an exacerbation, at which time a visit with a study doctor was arranged to confirm relapse.

Tissue microarrays and immunohistochemistry

Tissue microarrays were designed as described previously [18] by using two 0.6-mm tissue cores per case, taken from formalin-fixed, paraffin-embedded archival biopsy blocks, along with different controls, to ensure reproducibility and homogenous staining of the slides (Shanghai Biochip Co Ltd, Shanghai).

The antibody choice was empirical, based on availability and suitability for paraffin-embedded archival material. Immunohistochemistry was done using DAKO Envision™ System in the autoimmunostainer (Dako Autostainer, Copenhagen, Denmark). Primary antibodies used in this study included CD27 (1:50, NeoMarkers), CD70 (1:50, R&D), TRAF2 (1:50, Santa Cruz), TRAF3 (1:200, Santa Cruz), TRAF4 (1:50, Santa Cruz), TRAF5 (1:50, Santa Cruz), STAT3 (1:1200, Cell signaling), CD40 (1:100, Abcam), CD154 (1:100, R&D), CD80 (1:750, Abcam). Immunostains were scored semiquantitatively by two pathologists. Only protein expression profiles in intestinal epithelia were evaluated. Disagreements between the two pathologists were resolved with a multihead microscope. Higher score was considered as a final score in case of a difference between duplicate tissue cores. Scoring was finally determined without knowledge of patients' information. Results were scored by the quick score as previously described. [19] For methodologic reasons, quick scores were reformatted (positive to negative scores) into a format suitable for unsupervised analyses [20].

Data analysis and statistical methods

Hierarchical clustering and k-means clustering were applied to determine the classification of protein panel. Data of quick scores were reformatted as follows: -2 designated negative staining, 2 positive staining. Missing data was left blank in scored document. We used the Cluster 3.0 (average linkage, Pearson correlation) to classify CD, UC and nonspecific colitis. Results were displayed with TreeView. Distributions of protein markers and categorical variables were compared using chi-square tests. Kappa statistic was used to assess agreement in classification of cases based on expressions of biomarkers. Multiple group comparisons were applied by one-way ANOVA and followed posthoc analysis when significantly different.

Next, all variables were analyzed for their association with relapse using binary logistic regression. Time zero was defined as study entry, and patients were followed-up to relapse or to one year or the date of early withdrawal. All continuous predictors were analyzed for logistic regression. All patients were censored at one year of

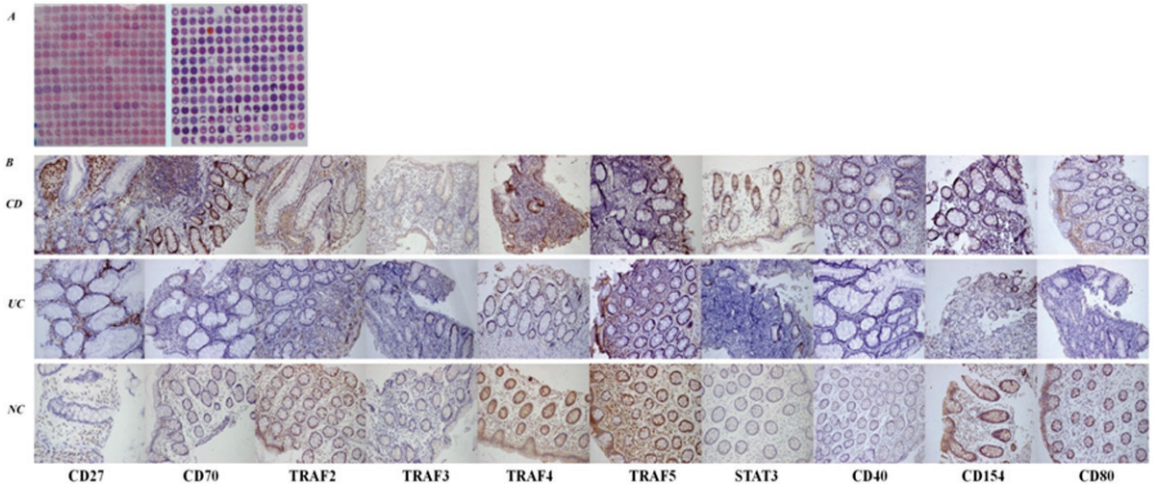

Figure 1. Protein expression profiles studied by immunohistochemistry on tissue microarrays. A. HE stain of paraffin blocks with 0.6 mm tissue cores. B. Examples of immunohistochemistry staining for 10 proteins. Magnification ×200 or ×400. CD, Crohn's disease; UC, ulcerative colitis; NC, nonspecific.

follow-up, death or relapse. We performed Spearman's rank correlation coefficient to assess the relationship among endoscopic disease activity indices and clinicopathologic characteristics.

SPSS for Windows version 13.0 (Chicago, IL) was used for statistical analysis of the data. Data are presented either with their 95% confidence intervals (95% CI) or median and 25%-75% percentile. Statistical tests were two-sided at the 5% level of significance.

Results

Different protein expression profile in patients with IBD and nonspecific colitis

A total of 120 patients with CD, 117 patients with UC and 120 patients with nonspecific colitis entered the present study. 78 patients with CD and 65 patients with UC relapse within one year of follow-up. Characteristics of enrolled subjects were shown in **Table 1**.

The expression of ten proteins was studied by immunohistochemistry using tissue microarrays. The quick scores of staining for all antibodies were heterogeneous among patients with CD, UC or nonspecific colitis (**Table 2**). Examples of staining are shown in **Figure 1**. Multiple comparisons indicated that quick score only showed minor significance in CD40 between patients with IBD and patients with nonspecific colitis (P=0.085, P≤0.2).

Unsupervised classification of protein expression profile

First, unsupervised hierarchical clustering analysis with average linkage was applied to the dataset of ten biomarkers. Proteins were ordered on the horizontal axis and samples were on the vertical axis based on similarity of expression profiles (**Figure 2**). Unsupervised hierarchical clustering analysis did not produce a dendrogram with well-defined cluster groups as CD, UC and nonspecific colitis (**Figure 2A**). Only a trend toward classification of IBD and nonspecific colitis was identified (**Figure 2A**, tree image). There was a significant concordance between cluster groups based on immunostaining data of TMA and clinical classification in distinguishing IBD from nonspecific colitis (kappa=0.498, P<0.001). Although the combined protein expression patterns in IBD cluster could hardly be subdivided to CD cluster and UC cluster because cases were scattered, we tried to figure out the trend to subgroup CD (**Figure 2B**) and UC (**Figure 2C**). However, protein expression patterns in IBD clusters showed a low concordance to subclassify UC from IBD (kappa=0.395, *P*<0.001). The combined protein expression patterns could not define a CD cluster (kappa=-0.051, P=0.474).

Second, in the present study, IBD cluster could not be clearly subdivided to a CD cluster and a UC cluster with hierarchical clustering analysis. Thus, we then analyzed the correlations

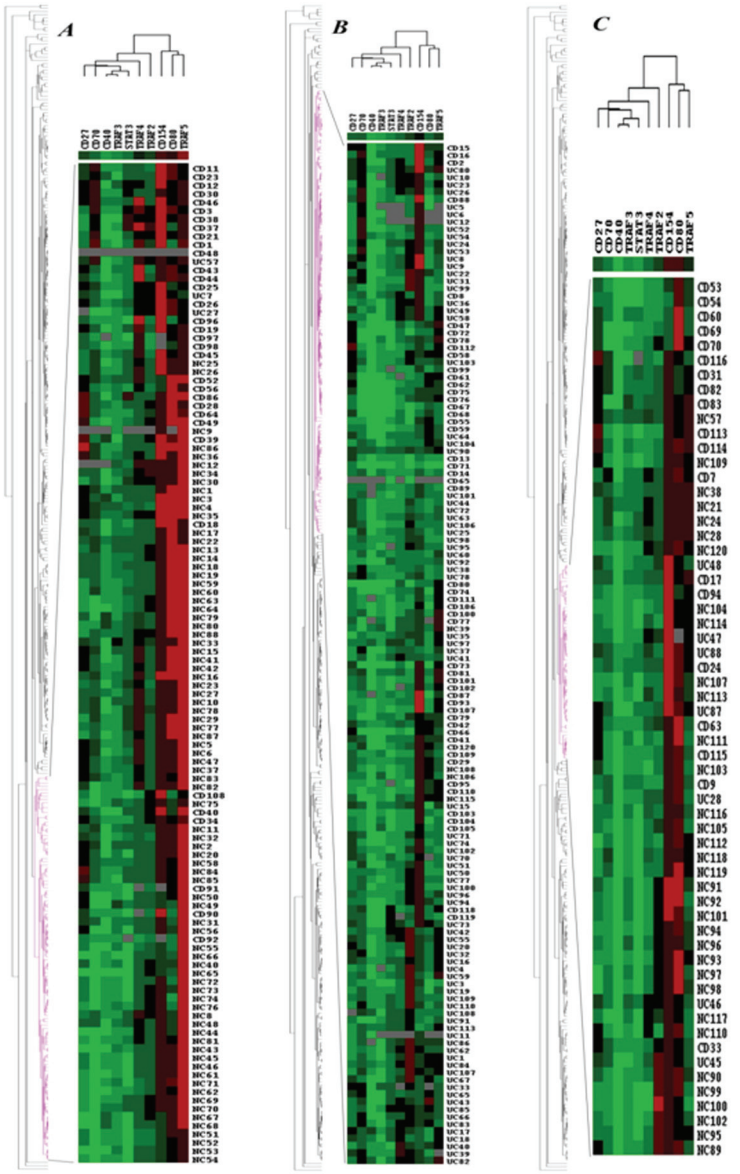

Figure 2. Hierarchical clustering analysis of protein expression profiles in nonspecific colitis (A), Crohn's disease (B) and ulcerative colitis (C) as measured by tissue microarray. Graphical representation of hierarchical clustering results based on expression profiles of proteins. Rows, samples; columns, proteins. Protein expression scores are depicted according to a color scale: red, positive staining; green, negative staining; black, zero; gray, missing data. Dendrograms of samples (to the left of matrix) and proteins (above matrix) represent overall similarities in expression profiles. In the dendrogram, the length of branch between two elements reflects their degree of relatedness. A trend towards cluster of nonspecific colitis (purple dendrograms to the left of matrix, zoomed in A) is shown to classify patients with inflammatory bowel disease and nonspecific colitis. Two major protein clusters are identified (above matrix).

between clinical classifications and expression profiles with k-means clustering analysis and chi-square tests. Similarly, no significance of overall concordance was indicated between three cluster groups based on k-means clustering analysis and clinical classification of CD, UC and nonspecific colitis (kappa=0.045, P=0.223).

Unsupervised hierarchical clustering analysis also found two major protein clusters that were clearly identified (**Figure 2**, above dendrogram). Despite heterogeneous expression, such analysis and color display highlighted groups of correlated proteins across correlated samples. CD27, CD70, CD40, TRAF3, TRAF4 and TRAF2 presented similar immunostaining feature, which was different from cluster of CD154, CD80 and TRAF5.

Supervised analysis of protein expression profile found factors associated with endoscopic disease activity index and relapse

Seven cases with CD were excluded from SES-CD evaluation because only small bowel disease was involved. When Spearman's rank correlation coefficient was used to assess the relationship between endoscopic disease activity indices and protein expression profiles, no significant association was indicated between protein expression profiles and endoscopic disease activity in patients with IBD (all P>0.05).

Logistic regression exploring possible interactions among clinicopathologic variables showed that only higher expression of TRAF2 was a predictor of relapse in patients with UC (P=0.006).

Discussion

When suspecting IBD, colonoscopy with biopsy is crucial for diagnosis and evaluation. In fact, histopathological reports often mention a diagnosis of nonspecific colitis, which is hard to separate colitis with similar histological patterns but distinct distribution patterns. Although nonspecific disease states are recognized, histological patterns can reflect pathogenesis, severity and duration. Furthermore, therapeutic decisions can be directed more appropriately if endoscopy and biopsy can reliably distinguish IBD from similar symptoms caused by other inflammatory or non-inflammatory disease in intestine, or if one could distinguish CD from UC [21]. In addition, few specific pathologic markers in IBD could help monitor response in the clinic or in clinical trials. Moreover, reliable prediction of the recurrence would help appropriate therapy to those who would most likely benefit from it and avoid the excessive maintenance therapy in patients with a low potential of relapse. Thus, in the present study, protein expression profiles are used as a framework to show patterns in classification, endoscopic assessment and prognosis in IBD patients.

Protein expression profiles distinguish IBD and nonspecific colitis

The diagnostic differentiation between IBD and nonspecific colitis is sometimes difficult. Moreover, in the search for molecular markers in IBD, individual markers that are specific and sensitive enough to differentiate between CD and UC are still lack [22]. In IBD, different pathways are activated, leading to the immune intolerance of normal intestinal flora. Thousands of protein networks are involved in the pathogenicity. Thus, clinicians should consider a panel of biomarkers for the differentiation, management and follow-up of IBD patients [23]. This study reflects our concerns over mucosal biopsy assessment of colitis and its role in accurately addressing the differentiation of IBD. We find that IBD and nonspecific colitis can be distinguished by tissue microarray. However, tissue microarray of IBD cluster could not be clearly subdivided to a CD cluster and a UC cluster. Low-grade inflammation under endoscopy is often reported as nonspecific colitis, which can be confusing to clinicians. Pathologic report of inflammation may be missed without biopsy of intestinal mucosa that appears normal during endoscopy [24]. On the other hand, microscopic colitis also presents essentially normal endoscopy but with histologic inflammation of colonic mucosa [25]. Alternatively, both acceptance and ignorance of all nonspecific colitis report as being a clinically significant diagnosis may lead to inappropriate management. Increased microscopic inflammation of the intestine is also present in healthy individuals [26], which should be carefully distinguished with IBD. However, colonic CD may be difficult to distinguish from UC on endoscopy or microscopic examination of biopsy samples [27]. CD and UC have significant overlap in mucosal immunity, which leads to consider multiple ways to distinguish them such via serology and gene expression.

Another finding from the present study is that TRAFs and their associated pathways can be divided into different groups based on diverse protein expressions. Cluster designed microarrays show that similar gene expression patterns indicate similar function [28]. It has been shown that TRAFs participate in the activation of NF-κB, JNK and MAPK pathways by recruiting CD27, CD30, CD40 or CD80 pathways [29]. It is clear that TRAFs have individually specific functions or act redundantly in transmitting signals via different receptors [30]. In particular, all the biomarkers in this study can be implicated for a better understanding of the mechanism regulating canonical or non-canonical NF-κB activation [31]. Although additional studies are required to clarify the exact mechanism of clustered proteins in IBD, we suspect that the clustered proteins are potentially involved in the similar signaling cascades.

Protein expression profiles predict relapse

Assessment of disease activity in patients with CD and UC is important both in clinical practice and in clinical trials. The importance of evaluating endoscopic disease activity in the long-term management of IBD is to distinguish quiescent from active disease and to establish mucosal healing [32, 33]. Serological markers such as acute phase reactants, cytokines and adhesion molecules, and fecal markers such as calprotectin and lactoferrin have been studied for assessment of disease activity [34]. However, the correlation between the clinical indices of activity and endoscopy or histology is variable

[35]. In our present study, it is found that protein expression profiles do not show a trend of association with endoscopic disease activity. The reason for such discrepancy is not clear yet and a subjective impression of endoscopic scoring results of IBD may be the reason. To date, in the assessment of the severity of intestinal mucosa inflammation in patients with IBD, serological and fecal markers also show controversial correlations with endoscopic disease activity [36, 37]. Although the cytokines, cytokines receptors and/or cytokine transcripts have been studied in the intestinal mucosa and have been found to be elevated correlating with endoscopic disease activity [38, 39], combination of immunomarkers in intestinal mucosa, endoscopic disease activity index and clinical activity score might be used simultaneously as assessment of disease severity in patients with IBD.

Diverse biological markers involved in the pathogenesis of IBD have been proposed as predictors of recurrence after treatment [40]. Recently, studies focusing on predicting disease relapse within the first year are emerging [41]. Several studies have indicated that higher concentration of fecal calprotectin might be a predictor of disease relapse within 12 months [42, 43]. We find that higher expression of TRAF2 is a predictor of relapse in UC patients within 12 months. By recruiting TCR-related intracellular molecules into the TRAF2 complex or regulated by costimulatory molecules, TRAF2 provides the T cell with a high level of NF-κB activity [44]. TNF-α induces the ubiquitination of TRAF2 to increase NF-κB-inducing kinase (NIK) phosphorylation. Consequently, the non-canonical pathway of NF-κB is activated [45]. However, we can't ignore that controversy exists regarding the importance of serological, fecal and immunostaining markers in determining relapse in IBD. Preliminary data from few adequately powered prospective studies and varying definitions of relapse may be the explanations for the controversy.

Conclusions

Although more repeated studies of a longer follow-up on a larger series of IBD patients is required. Our study indicates that protein expression profiles may be a clinically useful approach to show some patterns in classification and prognosis in patients with IBD.

Acknowledgments

Supported by grants from National Natural Science Foundation of China (No. 81000161 and No.81170362), Shanghai Jiao-Tong University School of Medicine Technology Fund (No. YZ1036). We would like to appreciate Dr. Lindholm Christopher from University of Chicago revised the language and discussion of this paper.

Conflict of interest statement

None.

Address correspondence to: Dr. Zhi-hua Ran, Division of Gastroenterology and Hepatology, Shanghai Jiao-Tong University School of Medicine Renji Hospital, Shanghai Institute of Digestive Disease; Key Laboratory of Gastroenterology & Hepatology, Ministry of Health (Shanghai Jiao-Tong University), 1630# Dongfang Road, Shanghai 200127, P R China. Phone: +86 21 63260930; Fax: +86 21 63266027; E-mail: ranzhihua62@sina.cn

References

[1] Carpenter HA, Talley NJ. The importance of clinicopathological correlation in the diagnosis of inflammatory conditions of the colon: histological patterns with clinical implications. Am J Gastroenterol 2000; 95: 878-896.

[2] Schulze HA, Häsler R, Mah N, Lu T, Nikolaus S, Costello CM, Schreiber S. From model cell line to in vivo gene expression: disease-related intestinal gene expression in IBD. Genes Immun 2008; 9: 240-248.

[3] Henriksen M, Jahnsen J, Lygren I, Sauar J, Schulz T, Stray N, Vatn MH, Moum B, Ibsen Study Group. Change of diagnosis during the first five years after onset of inflammatory bowel disease: results of a prospective follow-up study (the IBSEN Study). Scand J Gastroenterol 2006; 41: 1037-1043.

[4] Mowat C, Cole A, Windsor A, Ahmad T, Arnott I, Driscoll R, Mitton S, Orchard T, Rutter M, Younge L, Lees C, Ho GT, Satsangi J, Bloom S; IBD Section of the British Society of Gastroenterology. Guidelines for the management of inflammatory bowel disease in adults. Gut 2011; 60: 571-607.

[5] Casellas F, Barreiro de Acosta M, Iglesias M, Robles V, Nos P, Aguas M, Riestra S, de Francisco R, Papo M, Borruel N. Mucosal healing restores normal health and quality of life in patients with inflammatory bowel disease. Eur J Gastroenterol Hepatol 2012; 24: 762-769.

[6] Geboes K, Colombel JF, Greenstein A, Jewell DP, Sandborn WJ, Vatn MH, Warren B, Riddell RH; Pathology Task Force of the International Organization of Inflammatory Bowel Diseases. Indeterminate colitis: a review of the concept-what's in a name? Inflamm Bowel Dis 2008; 14: 850-857.

[7] Ghosh S, D'Haens G, Feagan BG, Silverberg MS, Szigethy EM. What do changes in inflammatory bowel disease management mean for our patients? J Crohns Colitis 2012; 6 Suppl 2: S243-S249.

[8] Moum B. Medical treatment: does it influence the natural course of inflammatory bowel disease? Eur J Intern Med 2000; 11: 197-203.

[9] Romberg-Camps MJ, Dagnelie PC, Kester AD, Hesselink-van de Kruijs MA, Cilissen M, Engels LG, Van Deursen C, Hameeteman WH, Wolters FL, Russel MG, Stockbrügger RW. Influence of phenotype at diagnosis and of other potential prognostic factors on the course of inflammatory bowel disease. Am J Gastroenterol 2009; 104: 371-383.

[10] D'Incà R, Dal Pont E, Di Leo V, Benazzato L, Martinato M, Lamboglia F, Oliva L, Sturniolo GC. Can calprotectin predict relapse risk in inflammatory bowel disease? Am J Gastroenterol 2008; 103: 2007-2014.

[11] Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. Gastroenterology 2000; 119: 15-22.

[12] Vermeire S, Van Assche G, Rutgeerts P. Laboratory markers in IBD: useful, magic or unnecessary toys? Gut 2006; 55: 426-431.

[13] Foley KF, Kao P. Biomarkers for inflammatory bowel disease. Clin Lab Sci 2007; 20: 84-88.

[14] Wang Q, Gong L, Dong R, Qiao Q, He XL, Chu YK, Du XL, Yang Y, Zang L, Nan J, Lin C, Lu JG. Tissue microarray assessment of selenoprotein p expression in gastric adenocarcinoma. J Int Med Res 2009; 37: 169-174.

[15] Bendardaf R, Buhmeida A, Hilska M, Laato M, Syrjänen S, Syrjänen K, Collan Y, Pyrhönen S. VEGF-1 expression in colorectal cancer is associated with disease localization, stage, and long-term disease-specific survival. Anticancer Res 2008; 28: 3865-3870.

[16] Daperno M, D'Haens G, Van Assche G, Baert F, Bulois P, Maunoury V, Sostegni R, Rocca R, Pera A, Gevers A, Mary JY, Colombel JF, Rutgeerts P. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointest Endosc 2004; 60: 505-512.

[17] Baron JH, Connell AM, Lennard JE. Variation between observers in describing mucosal appearances in proctocolitis. Br Med J 1964; 1: 89-92.

[18] Fernebro E, Dictor M, Bendahl PO, Fernö M, Nilbert M. Evaluation of the tissue microarray technique for immunohistochemical analysis in rectal cancer. Arch Pathol Lab Med 2002; 126: 702-705.

[19] Walker RA. Quantification of immunohistochemistry--issues concerning methods, utility and semiquantitative assessment I. Histopathology 2006; 49: 406-410.

[20] Jacquemier J, Ginestier C, Rougemont J, Bardou VJ, Charafe-Jauffret E, Geneix J, Adélaïde J, Koki A, Houvenaeghel G, Hassoun J, Maraninchi D, Viens P, Birnbaum D, Bertucci F. Protein expression profiling identifies subclasses of breast cancer and predicts prognosis. Cancer Res 2005; 65: 767-779.

[21] Papachristou GI, Plevy S. Novel biologics in inflammatory bowel disease. Gastroenterol Clin North Am 2004; 33: 251-269.

[22] Zhang T, Song B, Zhu W, Xu X, Gong QQ, Morando C, Dassopoulos T, Newberry RD, Hunt SR, Li E. An ileal Crohn's disease gene signature based on whole human genome expression profiles of disease unaffected ileal mucosal biopsies. PLoS One 2012; 7: e37139.

[23] Roda G, Caponi A, Benevento M, Nanni P, Mezzanotte L, Belluzzi A, Mayer L, Roda A. New proteomic approaches for biomarker discovery in inflammatory bowel disease. Inflamm Bowel Dis 2010; 16: 1239-1246.

[24] da Silva JG, De Brito T, Cintra Damião AO, Laudanna AA, Sipahi AM. Histologic study of colonic mucosa in patients with chronic diarrhea and normal colonoscopic findings. J Clin Gastroenterol 2006; 40: 44-48.

[25] Warren BF, Edwards CM, Travis SP. 'Microscopic colitis': classification and terminology. Histopathology 2002; 40: 374-376.

[26] Paski SC, Wightman R, Robert ME, Bernstein CN. The importance of recognizing increased cecal inflammation in health and avoiding the misdiagnosis of nonspecific colitis. Am J Gastroenterol 2007; 102: 2294-2299.

[27] Quinn PG, Binion DG, Connors PJ. The role of endoscopy in inflammatory bowel disease. Med Clin North Am 1994; 78: 1331-1352.

[28] Liu AY, Vêncio RZ, Page LS, Ho ME, Loprieno MA, True LD. Bladder expression of CD cell surface antigens and cell-type-specific transcriptomes. Cell Tissue Res 2012; 348: 589-600.

[29] Nakano H, Sakon S, Koseki H, Takemori T, Tada K, Matsumoto M, Munechika E, Sakai T, Shirasawa T, Akiba H, Kobata T, Santee SM, Ware CF, Rennert PD, Taniguchi M, Yagita H, Okumura K. Targeted disruption of Traf5 gene causes defects in CD40- and CD27-mediated lymphocyte activation. Proc Natl Acad Sci U S A 1999; 96: 9803-9808.

[30] Ha H, Han D, Choi Y. TRAF-mediated TNFR-family signaling. Curr Protoc Immunol 2009 Nov; Chapter 11: Unit11.9D.
[31] Sun SC. Non-canonical NF-κB signaling pathway. Cell Res 2011; 21: 71-85.
[32] Frøslie KF, Jahnsen J, Moum BA, Vatn MH; IBSEN Group. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. Gastroenterology 2007; 133: 412-422.
[33] Baert F, Moortgat L, Van Assche G, Caenepeel P, Vergauwe P, De Vos M, Stokkers P, Hommes D, Rutgeerts P, Vermeire S, D'Haens G; Belgian Inflammatory Bowel Disease Research Group; North-Holland Gut Club. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. Gastroenterology 2010; 138: 463-468.
[34] Desai D, Faubion WA, Sandborn WJ. Review article: biological activity markers in inflammatory bowel disease. Aliment Pharmacol Ther 2007; 25: 247-255.
[35] Geboes K. Pathology of inflammatory bowel diseases (IBD): variability with time and treatment. Colorectal Dis 2001; 3: 2-12.
[36] Canani RB, Terrin G, Rapacciuolo L, Miele E, Siani MC, Puzone C, Cosenza L, Staiano A, Troncone R. Faecal calprotectin as reliable non-invasive marker to assess the severity of mucosal inflammation in children with inflammatory bowel disease. Dig Liver Dis 2008; 40: 547-553.
[37] Peterson CG, Sangfelt P, Wagner M, Hansson T, Lettesjö H, Carlson M. Fecal levels of leukocyte markers reflect disease activity in patients with ulcerative colitis. Scand J Clin Lab Invest 2007; 67: 810-820.
[38] Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. Am J Gastroenterol 2008; 103: 162-169.
[39] Schmidt C, Giese T, Ludwig B, Mueller-Molaian I, Marth T, Zeuzem S, Meuer SC, Stallmach A. Expression of interleukin-12-related cytokine transcripts in inflammatory bowel disease: elevated interleukin-23p19 and interleukin-27p28 in Crohn's disease but not in ulcerative colitis. Inflamm Bowel Dis 2005; 11: 16-23.
[40] Pardi DS, Sandborn WJ. Predicting relapse in patients with inflammatory bowel disease: what is the role of biomarkers? Gut 2005; 54: 321-322.
[41] Consigny Y, Modigliani R, Colombel JF, Dupas JL, Lémann M, Mary JY; Groupe d'Etudes Thérapeutiques des Affections Inflammatoires Digestives (GETAID). A simple biological score for predicting low risk of short-term relapse in Crohn's disease. Inflamm Bowel Dis 2006; 12: 551-557.
[42] Costa F, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, Ricchiuti A, Marchi S, Bottai M. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut 2005; 54: 364-388.
[43] Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. Gastroenterology 2000; 119: 15-22.
[44] So T, Soroosh P, Eun SY, Altman A, Croft M. Antigen-independent signalosome of CARMA1, PKCθ, and TNF receptor-associated factor 2 (TRAF2) determines NF-κB signaling in T cells. Proc Natl Acad Sci U S A 2011; 108: 2903-2908.
[45] Bhattacharyya S, Dudeja PK, Tobacman JK. Tumor necrosis factor alpha-induced inflammation is increased but apoptosis is inhibited by common food additive carrageenan. J Biol Chem 2010; 285: 39511-39522.