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Colonic expression of Runx3 protein and TGF- β_1 and their correlation in patients with irritable bowel syndrome

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ABSTRACT

Objective: To investigate the role of Runx3 protein and TGF- β_1 in the pathogenesis of irritable bowel syndrome (IBS), as well as the correlation of these two proteins. **Methods:** Colonic tissue was collected from patients with IBS and normal persons. The colonic expression of Runx3 protein and TGF- β_1 was detected with immunohistochemistry method. Semi-quantitative analysis was used to evaluate the staining degree of these two proteins. **Results:** Compared with their counterparts, patients with IBS did not show any changes in the colonic expression of Runx3 protein and TGF- β_1 ($P>0.05$). Interestingly, there was a significant correlation between Runx3 protein and TGF- β_1 in patients with IBS ($P<0.05$). **Conclusions:** The role of Runx3 protein and TGF- β_1 in the pathogenesis of IBS remains to be further studied.

1. Introduction

Irritable bowel syndrome (IBS) is one kind of the dysfunctional intestinal diseases with the characteristic of bellyache, abdomen bulge accompanied with the changing defecating habit and stool character. The precise mechanism underlying this disease remains unclear. Recently, more and more researchers accepted the concept that IBS is a syndrome of dynamical and sensory abnormality induced by multiple factors. It was reported that some patients with IBS show significant inflammation and/or immunological disturbance in their colon[1].

As a novel tumor-suppressor gene, Runx3 was recently proved to play an important role in the development process of immunocytes, especially that of T lymphocyte and dendritic cells[2,3]. Runx3 gene knockout mice

show spontaneous inflammation in their colon with the characteristic of the abundant Th1 cells mixed with a few Th2 cells[4].

TGF- β_1 could modulate the growth and differentiation of various immunocytes and non-immunocytes, thus could suppress the intestinal inflammation[2,3]. As an important transcription modulator in the signal transduction pathway of TGF- β , Runx3 could activate smad protein and regulate the transcription of its target gene by TGF- β_1 , thus exert its impact on the modulation of the development and differentiation of various cells including epithelial cells and immunocytes.

The current study aimed to investigate the expression of Runx3 protein and TGF- β_1 in the colon from patients with IBS, as well as the relationship of these two proteins in the pathogenesis of this disease.

2. Materials and methods

2.1. Subjects and reagents

A total of 74 patients were selected, who were confirmed

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as IBS by Rome III standard^[5] and were admitted from July to October, 2010 at the clinic of digestive diseases, Hainan Provincial People's Hospital. All patients were evaluated by colonic–rectum endoscopy and showed normal colonic tissue. Meanwhile, 51 healthy volunteers were selected as a control group.

Rat–anti–human Runx3 monoclonal antibodies were purchased from Tiancheng Corp, Shanghai, China; Rat–anti–human TGF- β_1 monoclonal antibodies purchased from Zhongshan Corp, Beijing, China; PV-6001 two–step kit and DAB colour–producing reagent kit purchased from Zhongshan Corp, Beijing, China.

2.2. Histological studies

Colonic tissues were collected and fixed in 10% formalin. The slices were stained with hematoxylin–eosin and observed under light microscope for their histological changes.

2.3. Immunohistochemistry studies

Immunohistochemistry EnVision two–stage method was utilized to detect the expression of Runx3 protein and TGF- β_1 in colon tissue. Briefly, slices were treated as usual from dewax to washing followed by immersed in 3% H₂O₂ for 10 min, and washed by distilled water for three times. Primary antibodies (rat anti human Runx3 and TGF- β_1 monoclonal antibodies at working solution of 1:200 and 1:100, respectively) were used. PBS was taken to be the substitute for two primary antibodies as the negative control. The secondary antibody was rabbit–anti–rat IgG–HRP polymer. Antigens were repaired by microwaves in sodium citrate damping fluid (pH =6.0). The other procedures were operated strictly according to the manual.

The expression of Runx3 protein and TGF- β_1 in colon tissue was semi–quantitatively analyzed following the standard of both the percent of positive cells and the degree of stained cells as previously described by Yao *et al*^[6]. The percent of positive cells less than 1% was scored as 0, 2%–25% as 1, 26%–50% as 2, 51%–75% as 3, more than 75% as 4. For the degree of the staining, no staining was scored as 0, straw yellow as 1, palm yellow as 2, puce as 3. The total score was the percent multiplied by the staining degree ranging from 0 to 12. The total score of 0–1 was considered as negative (I), 2–4 as positive (II), 5–8 9–12 as strong positive (III; IV).

2.4. Statistical analysis

SPSS13.0 software was used for statistical analysis. Measurement data was expressed by mean \pm standard deviation, *t* test was used for group comparison. Spearman correlation analysis was utilized to investigate the correlativity of Runx3 protein and TGF- β_1 . *P*<0.05 was considered as being significant.

3. Results

3.1. Pathological changes of colon tissues

Neither tissue damage nor infiltration of inflammatory cells was found in the mucosa membrane of both normal control and IBS groups (Figure 1).

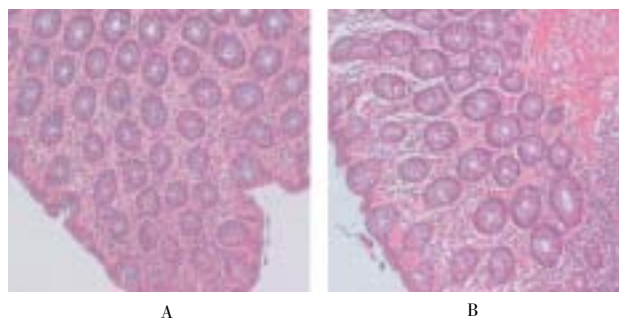


Figure 1. Pathological changes of the colon (HE \times 10). A: normal control, B: IBS patients.

3.2. Expression of Runx3 protein and TGF- β_1 in colon tissue

Runx3 protein and TGF- β_1 were expressed in colon tissue from two groups. Runx3 positive cells were mainly scattered among epithelium cells, gland cells and lamina propria cell, whose palm granule was located in the nucleus and sometimes in the plasma. The palm granule of TGF- β_1 was detected in the plasma and cell membrane (Figure 2). Analysis by Fisher definite probability method, positive percents of cells in both Runx3 protein and TGF- β_1 showed no significant differences in normal control and IBS groups (*P*>0.05) (Table 1).

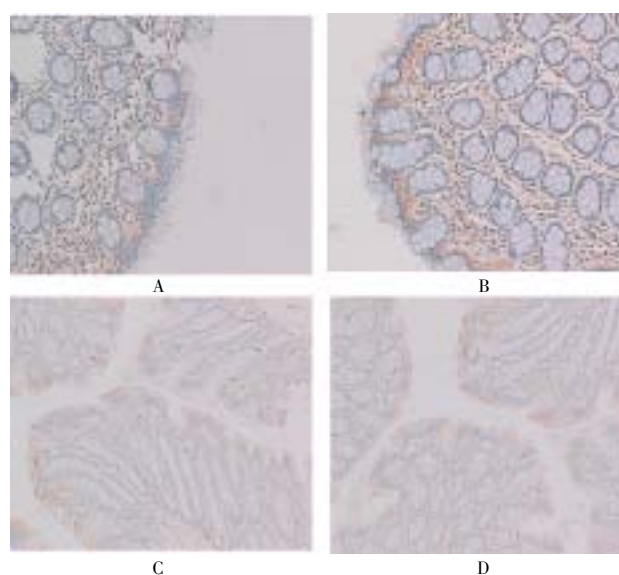


Figure 2. Expression of Runx3 protein and TGF- β_1 in colon tissue (40 \times).

A, B : Runx3 protein positive cells; C, D: TGF- β_1 positive cells.

Table 1Expression of Runx3 protein and TGF- β_1 protein in colon tissue (mean \pm SD).

	Group	Score of positive percent	Score of staining degree	Total score
Runx3	Control	2.23 \pm 1.09	1.77 \pm 1.09	4.81 \pm 3.95
	IBS	2.21 \pm 0.90	1.65 \pm 0.77	4.08 \pm 2.68
	Z	0.39	0.32	0.45
	P	0.70	0.75	0.65
TGF- β_1	Control	0.77 \pm 0.72	0.68 \pm 0.65	0.87 \pm 0.96
	IBS	0.91 \pm 0.74	0.81 \pm 0.61	1.14 \pm 1.30
	Z	0.78	1.06	0.77
	P	0.41	0.29	0.44

3.3. Correlativity of Runx3 protein and TGF- β_1 in colon tissue from patients with IBS

Spearman correlation analysis was utilized to investigate the correlativity of Runx3 protein and TGF- β_1 . There was a significant correlation in score of positive percent ($r=0.30$, $P=0.002$), score of staining degree ($r=0.29$, $P=0.003$) and total score ($r=0.35$, $P=0.0002$) between Runx3 protein and TGF- β_1 in colon tissue from IBS patients.

4. Discussion

It is well known that multiple factors including abnormal dynamics, the hypersensitive visceral sensation, and the light inflammation are involved in the pathogenesis of IBS. Recently, the interest was focused on the role of the intestinal inflammation in IBS[1]. Weersma and Guo *et al*[7,8] reported that colonic expression of Runx3 mRNA and Runx3 protein significantly increased in patients with ulcerative colitis. Both Runx3 and TGF- β_1 participate in the processes of proliferation, differentiation and inflammation by immunocytes[2-4]. However, the expression of these two proteins and their correlativity remains unclear in patients with IBS.

As one kind of regulative factor in the signal transmitting pathway of TGF- β , Runx3 gene could induce the inhibitory effect of TGF- β_1 on the mature process of the dendritic cells (DC)[4], which is involved in the gut inflammation. Stadnicki *et al*[9] reported that the TGF- β_1 level in the muscular layer continuously increased in PI- IBS, lasting for at least one month, and that the increased TGF- β_1 could enhance the smooth muscle's excitability.

Similar to the result of our previous study[10] on rat model with IBS, the colonic level of Runx3 protein and TGF- β_1 did not show any remarkable changes in IBS patients compared with their control counterparts. This negative data maybe result from the clinic type of IBS, the lasting time of the inflammation and the depth of the samples.

But interestingly, the colonic level of Runx3 protein and TGF- β_1 in patients with IBS show a significant positive correlation, suggesting that Runx3- TGF- β_1 inflammation pathway could be involved in human IBS. This kind of condition is not reported by any other authors. It remain unclear that if and how Runx3, one of the key regulative genes modulating the development and function of intestinal DC, could exert its impact on the intestinal DC's behavior,

thus regulate the intestinal inflammation and immunity.

Conflict of interest statement

We declare that we have no conflict of interest.

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