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Study on the expression of Runx3 and TGF-β$_1$ protein in the colonic tissue from rats with irritable bowel syndrome

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ABSTRACT

Objective: To investigate the expression of Runx3 and TGF-β$_1$ protein in the colon from rats with irritable bowel syndrome (IBS). **Methods:** Rat model for IBS was established by intracolonic instillation with acetic acid and restraint stress methods, which was confirmed by determinating the visceral sensitivity of the animals, including abdominal withdrawal reflex (AWR) score and the electronic behavior of the abdomen wall. The rats were randomly assigned into three groups: IBS$_1$ group (restraint stress, n=25); IBS$_2$ group (both instillation with acetic acid and restraint stress, n=25) and Control group (n=16). The colonic tissue samples were collected for histological study and the expression of Runx3 and TGF-β$_1$ proteins were detected by immunohistochemistry. Meanwhile, the relationship of these two proteins was calculated. **Results:** Visceral hypersensitivity (AWR and abdominal electrical activity) was significantly enhanced in IBS$_1$ and IBS$_2$ groups than other groups. The colon tissue in all groups did not show any signs of inflammation. Furthermore, the expression of Runx3 and TGF-β$_1$ protein in the colon from all groups show no significant difference (P>0.05), with no remarkable relevancy between each other (P>0.05). **Conclusions:** The rat model for IBS was successfully established. We did not find any significant changes in the expression of Runx3 and TGF-β$_1$ protein in the colon tissue from IBS rats, suggesting that the quantitative changes may be not the way by which Runx3 and TGF-β$_1$ protein play their roles in IBS. The accurate roles of Runx3 and TGF-β$_1$ proteins 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 kind of disease remains unclear. Recently, more and more researchers accepted the concept that IBS is a syndrome of dynamical and sensory abnormity 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, thus exert its impact on the modulation of the development and differentiation of various cells including epithelial cells and immunocytes by TGF-β.

The current study aimed to investigate the expression of Runx3 and TGF-β$_1$ proteins in the colon from rats with IBS,

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as well as the roles of these proteins in the pathogenesis of this disease.

2. Material and methods

2.1. Animals and grouping

Adult male Wistar rats, weighting about 200 g, purchased from the Center for Diseases Control of Hunan Province, were randomly assigned into 4 groups: IBS_1 group (restraint stress, n=15); IBS_2 group (both instillation with acetic acid and restraint stress, n=15); Control group (n=15).

2.2. Main reagents and apparatus

Rabit-anti-rat Runx3 multiple clone antibodies purchased from Tiancheng Corp, Shanghai, China; PV-6001 two-step Kit and DAB color-producing reagent Kit purchased from Zhongshan Corp, Beijing, China; multiple-tract biological signal gathering and processing system (Type: RM6280C) and electrode (needle inserting type) purchased from Chengdu Instrument Plant. 8F catheter (diameter 2 mm, sacculus max volume 3 mL, max diameter 2 cm) was used as the sacculus distending duct intra-colon and rectum purchased from Kangkang Medical Limited Corp, Zhejiang, China).

2.3. IBS Modeling

The rats in IBS_1 group were treated for IBS model with restraint stress as previously described by Williams *et al*[5]. Briefly, the rats fasting for 24 h were anesthetized with aether and tied for 1 h (from their regaining consciousness). The animals in IBS_2 group were treated as described by La *et al*[6]. The animals were filled with 1ml acetic acid (40 mL/L) by their anus followed by washing with 1 mL PBS (0.01 mol/L). 7 d later, they were treated as their counterparts in IBS1 group.

The visceral sensitivity of the animals, including AWR score and the electronic behavior of the abdominal muscle was determined after sacculus dilation in their anuses[7,8]. Briefly, the animals in all groups were inserted by anus with 8F catheter with sacculus (upto 7.0 cm into the rectum), fixed to their tails. The rats were put into a transparent plastic chamber, fixed so that they could not move with freedom. 30 min later, the rat were dilated in their anus with sacculus for three times, with the volume of 1.0, 1.5, 2.0 mL respectively. The dilation last for 5 min at 30 sec interval. The times of the contraction by the abdomen wall were detected.

2.4. Histological studies

After the determination of the electronic behavior of the abdomen wall's muscle, the animals were sacrificed by injection of superfluous anesthetic, whose colonic tissues. After fixed in 10% formalin, the slices were studied under light microscope for their histological changes.

2.5. Immunohistochemistry studies

Immunohistochemisty EnVision two-stage method was utilized to detect the expression of Runx3 and TGF-β_1 in colon tissue. Briefly, the slices were treated as usual from dewax to washing followed by immersed in 3% H_2O_2 for 10 min and washing in distilled water for three times. The primary antibodies (rabbit anti rat Runx3 and TGF-β_1 multiple clonal antibodies at working solution of 1:400 and 1:100 respectively) were used. PBS was taken to be the substitute for the two primary antibodies as the negative control. EnVision kit was bought from Dako Company. 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 and TGF-β_1 in colon tissue was semi-quantitatively analyzed following the standard of both the percent of the positive cells and the degree of the stained cells as previously described by Yao *et al*[9]. The percent of the 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 and 9-12 as strong positive (Ⅲ; Ⅳ).

2.6. Statistical analysis

SPSS 13.0 software was used for statistical analysis. Measurement data was expressed by mean±standard deviation, t tests was used for group comparison. Spearman correlation analysis was utilized to investigate the correlativity of Runx3 and TGF-β_1. P<0.05 was considered as being significant.

3. Results

3.1. Comparison of the abdomen wall muscle's electronic behavior in various groups

Compared with their normal counterparts, the abdomen wall muscle's electronic behavior of the rats from all three IBS groups increased significantly (P<0.05), suggesting that there was visceral sensation hypersensitivity in these rats. Unfortunately, no significant difference was observed among the three IBS groups (P>0.05) (Figure 1).

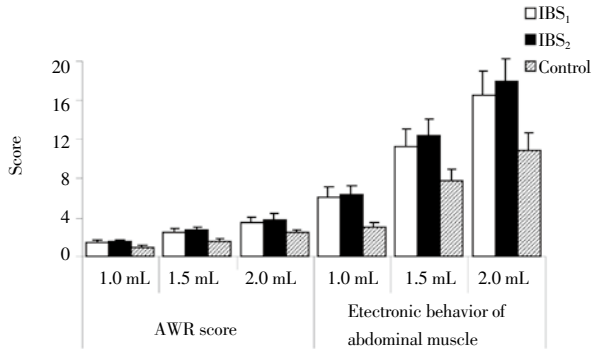

Figure 1. The AWR score and electronic behavior of the abdominal muscle in IBS rats.

3.2. Pathological changes of the colon tissues from IBS rats.

Neither tissue damage nor infiltration of inflammatory cells was found in the mucosa membrane from both normal control and IBS groups (Figure 2).

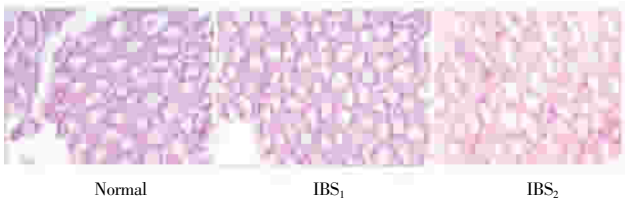

Figure 2. Pathological changes of the colon from IBS rats.

3.3. Expression of Runx3 and TGF-1 proteins in colon tissue

Runx3 and TGF-β_1 protein were found expressed in the colon tissue from all groups (Table 1 & 2). 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 protein was detected in the plasma and cell membrane (Figure 3). Analyzed with Fisher definite probability method, the positive percents of the cells expressing Runx3 and TGF-β_1 protein show no significant difference (P>0.05).

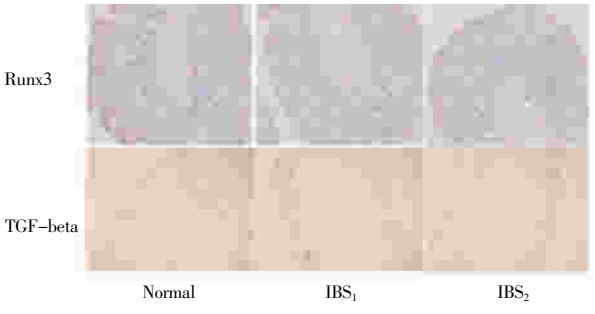

Figure 3. Expression of Runx3 and TGF-β_1 in colon tissue from IBS rats.
Runx3 and TGF-β_1 protein were found expressed in the colon tissue from all groups. The expression of Runx3 and TGF-β_1 protein show no significant difference among the two IBS groups and normal group.

Table 1
Expression of Runx3 protein in colon tissue from IBS rats.

Group	*n*	Score of positive percent	Score of staining degree	Total score
IBS_1	25	1.47±0.92	1.60±0.99	2.80±2.24
IBS_2	25	1.53±1.06	1.60±1.06	2.93±2.52
Control	16	1.60±0.99	1.33±0.98	2.67±2.24

Table 2
Expression of TGF-β 1 protein in colon tissue from IBS rats.

Group	*n*	Score of positive percent	Score of staining degree	Total score
IBS_1	25	1.53±1.06	1.46±0.83	2.27±2.66
IBS_2	25	1.27±0.96	1.47±1.06	1.53±1.46
Control	16	1.53±0.99	1.40±1.12	1.93±1.53

3.4. Correlativity of Runx3 and TGF-β_1

Spearman correlation analysis was utilized to investigate the correlativity of Runx3 and TGF-β_1. There was no significant correlation between the expression of Runx3 and TGF-β_1 protein in colon tissue from IBS rats (Table 3, P>0.05).

Table 3
The correlativity of Runx3 and TGF-β_1 in colon tissue from IBS rats.

Group	*n*	Correlation coefficient	*P* value
IBS_1	15	0.418	0.121
IBS_2	15	0.055	0.846
Control	15	0.051	0.856

4. Discussion

IBS is one kind of functional colonic diseases in which multiple and sophisticated factors are involved. The precise mechanism of this disease remains unclear, although a lot of studies suggest that it results from multiple factors such as the abnormal dynamics, the hypersensitive visceral sensation and the light inflammation. Recently, the interest was focused on the role of the intestinal inflammation in IBS[1,10]. Some proofs were reported such as increased mast cells, epithelial lymphocytes, $CD3^+$ cells, $CD25^+$ cells and microphages[1,11,12]. The intestinal inflammation underlying IBS may be associated with acute gastroenteritis and some genic factors[11,12].

In the current study, we firstly established the rat model for IBS with restraint stress and clysis with acetic acid[5]. Without any pathological changes in the colon from the rats in the restraint stress group, significant histological injure was observed, including edema, engorge and infiltrating inflammatory cells in the restraint stress plus clysis with acetic acid group. The rats in the two groups show the enhanced responsibility to the stimuli within rectum and colon.

As one of the cancer-suppressor genes and a member of the Runx framework region transcript factor family, Runx3 gene plays an important role in the process of functional differentiation of the T lymphocytes, especially cytotoxic

$CD8^+$ T lymphocytes, which exert their protective role in the intestinal mucosal immune barrier against exogenous pathogens[2,14,15]. Runx3 gene also modulates the mucosal immunity by down-regulating Th1/Th2 type immune response[4]. Our results show no changes of the expression of Runx3 protein in the colon from IBS rats, suggesting that the role of Runx3 gene in IBS could be much more complex than expected previously.

Runx3 gene could induce the inhibitory effect of TGF-β_1 on the mature process of the dendritic cells[16]. As a member of growth factor supper family, TGF-β_1 could participate in the pro-inflammation type IBS. In the current study, we did not find any remarkable pathological changes in the rectum and colon of the IBS rats. But we also did not find any abnormal expression of this kind of protein. Some authors reported that in the muscle layer from pro-inflammation type IBS, the expression of TGF-β_1 significantly increased, which could be involved in the pathogenesis of this type of IBS. On the other hand, Gonsalkorale *et al*[12] reported unchanged TGF-β_1 level in IBS, which was similar with our results. The point is that the quantity may not be parallel with the function. So the role of TGF-β_1 remains further study. On the other hand, the data from the animals does not always agree with that from patients, which still remains to be studied.

Conflict of interest statement

We declare that we have no conflict of interest.

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