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Runx3 might participate in regulating dendriti cell function in patients with irritable bowel syndrome

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ABSTRACT

Objective: To evaluate the expression levels and correlations among the expressions of transforming growth factor-β1 (TGF-β1), Runx3 and CD83 in colonic mucosal specimens from IBS patients. **Methods:** A total of 40 patients were selected, who were confirmed as IBS by Rome III standard and 40 healthy volunteers served as control. Colonic mucosal specimens of each subject were collected from colon sigmoideum with biopsy forceps. Runx3, TGF-β1, and CD83 (the marker for immunecompetent mature dendritic cells (DCs) mRNA in the sigmoid colon tissue were measured by real-time fluorescence quantitative PCR. **Results:** Compared with the control group, CD83 mRNA expressions were higher in patients with IBS than in healthy controls ($P<0.05$) and were associated with runt-related transcription factor 3 (Runx3) mRNA levels ($r=-0.361$, $P<0.05$). Meanwhile, Runx3 mRNA levels were associated with TGF-β1 mRNA expressions in irritable bowel syndrome (IBS) patients ($r=0.402$, $P<0.05$). However, there was no correlation between the mRNA expressions of TGF-β1 and CD83 ($P>0.05$). **Conclusions:** The increase of abnormal dendritic cells might influence the occurrence and development of IBS. TGF-β1 signal pathway might not be involved in Runx 3-regulated maturation of dendritic cells in IBS.

1. Introduction

Irritable bowel syndrome (IBS) is a common intestinal disorder characterized by persistent or intermittent abdominal pain or discomfort,distention,and changes in stool pattern. It has been became a serious health problem that affacts an estimated 2.9%-15.6% of people in Asian countries nowadays[1]. IBS is associated with abnormal intestinal motion and sensations,intestinal infection,hypothalamic-pituitary-gut axis dysregulation. Recently, a growing number of findings proved that immune activation play a majoy role in the pathophysiology of IBS[2]. The intestinal mucosa contains numerous DCs, which can mediate the innate and the adaptive immune system[3,4].

Transforming growth factor-β1 (TGF-β1) belongs to a well-defined multipotent cytokine family known to regulate several pathophysiological events and exhibit the broadest spectrum of biological activities. Runt-related transcription factor 3 (Runx 3) is a novel tumor-suppressor gene,it involved in the differentiation of immune cells[5], especially the each step of T-cell differentiation[6]. As a part of the innate immune response, Runx3, TGF-β1 and dendriti cells have been postulated to participate in mucosal and systemic immune responses in many studies,but the relationship of them have not been reported. The aim of this study was to evaluate the expression levels and correlations among the expressions of TGF-β1, Runx3 and CD83 in colonic mucosal specimens from IBS patients.

2. Materials and methods

2.1. Subjects and specimens

A total of 40 patients were selected,who were confirmed as IBS by Rome III standard and were admitted from March to December, 2013 at the clinic of digestive diseases, Hainan Provincial People's Hospital (Haikou, China). All patients were evaluated by colonic-rectum endoscopy and showed normal colonic tissue. Meanwhile, 40 healthy volunteers were selected as control. Colonic mucosal specimens of

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each subject were collected from colon sigmoideum with biopsy forceps and specimens were preserved in stored at −80 ℃immediately for subsequent RNA extraction.

2.2. Real-time PCR

Total RNA was isolated from colonic tissue using the RNeasy Micro Kit (Qiagen, Santa Clarita, CA), reverse transcription was performed using the Power RT Kit cDNA (Bio TeKe, Beijing, China) according to the manufacturer's protocols. Real-time PCR was performed to measure expression levels of target mRNAs using a 2×SYBR realtime RT-PCR premixture (Bio TeKe, Beijing, China). Briefly, the reactions were incubated at 95 ℃ for 2 min, followed by 45 cycles of denaturation at 95 ℃ for 15 s, annealing at 60 ℃ for 60 s in ABI PRISM 7300 Sequence Detection System Thermal Cycler(Applied Biosystems). Oligonucleotides (Table 1) were designed by Primer 5 software and synthesised at the Sangon Biotech (Shanghai, China).

Table 1

Primers in this study.

Primers	Primer or probe sequence (5′to 3′)
Runx3	Forward: GACTGTGATGGCAGGCAATGA
	Reverse: CGAAGCGAAGGTCGTTGAA
TGF-β 1	Forward: GGGACTATCCACCTGCAAGA
	Reverse: CCACCCGGTCGCGGGTGCTGT
CD83	Forward: TCCATCCTCTCTTCACCACC
	Reverse: CTGTGCCCACCATATTCC
GAPDH	Forward: TTTGGTATCGTGGAAGGACTC
	Reverse: GTAGAGGCAGGGATGATGTTCT

2.3. Statistical analysis

Statistical analysis was performed using SPSS software version 17.0. Results are reported as means±SD Student's *t*-test was used to compare Runx3, TGF-β 1 and CD83 mRNA levels between the IBS and control group. Correlations between two data sets were assessed using Persons correlation test. *P*-values of <0.05 were considered significant.

3. Results

3.1. Runx3, TGF-β 1 and CD83 mRNA expressions in sigmoid colon tissue from IBS patients

Compared with the control group, CD83 mRNA expressions were higher in patients with IBS than in healthy controls ($P<0.05$) (Figure 1). However , there was no significance in colonic mRNA expressions of Runx 3 ($P>0.05$) and TGF-β 1 ($P>0.05$) when compared with the control group.

3.2. Correlations among mRNA expressions of TGF-β 1, Runx3 and CD83

CD83 mRNA expressions were associated with Runx3 mRNA levels ($r=-0.361$, $P<0.05$). Meanwhile, Runx3 mRNA levels were associated with TGF-β 1 mRNA expressions in IBS patients ($r=0.402$, $P<0.05$) (Figure 2). But there is no correlation between the mRNA expressions of TGF-β 1 and CD83 ($P>0.05$).

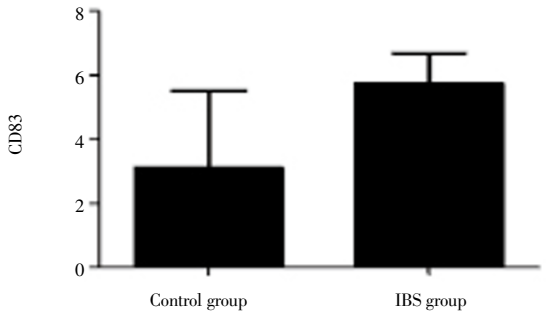

Figure 1. Runx3, TGF-β 1 and CD83 mRNA expressions in sigmoid colon tissue from IBS patients.

Compared with the control group, CD83 mRNA expressions were higher in patients with IBS than in healthy controls ($P<0.05$). However, there was no significance in colonic mRNA expressions of Runx 3 ($P>0.05$) and TGF-β 1 ($P>0.05$) when compared with the control group.

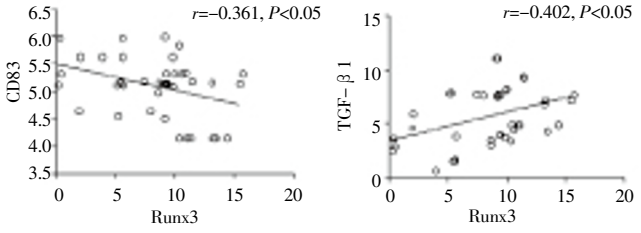

Figure 2. Correlations among mRNA expressions of TGF-β 1, Runx3 and CD83.

CD83 mRNA expressions were associated with Runx3 mRNA levels ($r=-0.361$, $P<0.05$). Meanwhile, Runx3 mRNA levels were associated with TGF-β 1 mRNA expressions in IBS patients ($r=0.402$, $P<0.05$). But there is no correlation between the mRNA expressions of TGF-β 1 and CD83 ($P>0.05$).

4. Discussion

IBS is a functional gastrointestinal disorder characterized by abdominal pain and altered bowel habits in the absence of specific and unique organic pathology. Its pathophysiology is still not entirely clear.Emerging evidence revealed that inflammation play a crucial role in the occurrence and development of IBS[7,8].

Dendritic cells (DCs) are key regulators in the immune system. DCs are found in two functionally distinct states: immature and mature dendritic cells. Immature DCs are present in peripheral tissues and are mainly phagocytic cells. Mature DCs are specialized antigen presentation cells that orchestrate innate and adaptive immune responses, they can act by priming abnormal T cell responses to the enteric flora in organized lymphoid tissues. Sustain DCs reactivity within the inflamed mucosa via the release of proinflammatory cytokines, for example, mature DCs expressing higher levels of costimulatory molecules (CD40,

CD80, and CD86) and increased amounts of IL-12p40 and IL-23p19 upon CD40 ligation[9]. While IL-12p40 and IL-23p19 can form IL-23, which is important for the growth and stabilization of Th17 cells in the mouse and their differentiation in humans[10], while Th17 cell differentiation can adrive inflammation. DCs play an important role in the occurrence and development of intestinal disease. Several observations suggest that DCs may play a pathogenic role in humans and in mouse models of inflammatory bowel diseases (IBDs), including Crohn disease and ulcerative colitis, are chronic relapsing inflammatory diseases of the gastrointestinal tract. Murakami *et al*[11] showed an increase of mucosal $CD83^+$ and $CD86^+$ cells producing macrophage inhibitory factor, which is thought to contribute to neutrophil recruitment and activation in ulcerative colitis. Cremon *et al*[12] demonstrated that the magnitude of the immune infiltrate detected in patients with IBS was markedly lower than that of active or quiescent ulcerative colitis in some comparative studies, this may imply that DCs also plays a role in intestinal inflammation in IBS patients.

Some research shows that TGF-β_1 can inhibit the maturation of DC. TGF-β_1 plays an important role in mediating the balance of the inflammation responses within the intestinal mucosa. Notably, TGF-β_1 promotes early dendritic cells' development in vitro and suppresses immature dendritic cells' activation and maturation[13]. For exampled treated with TGF-β_1 *in vitro* become less responsive to maturation stimuli such as IL-1β and TNF-α [14], thereby reducing the efficacy of dendritic cells to stimulate T lymphocytes.

The mammalian RUNX3 gene resides on human chromosome 1p36.1 and mouse chromosome 4 respectively. It belongs to the RUNX family of transcription factors, which contains three genes. In mice, Sugai *et al*[5] found that the loss of Runx3 in T cells resulted in suppression of Treg cell function which lead to the development of colitis in Runx3-/- animals.

Runx3 is highly expressed in DCs, where its functions as a component of TGF-β signaling cascade[15]. Runx3 KO DCs do not respond to TGF-β, their maturation is accelerated and accompanied by an increase defficacy to stimulate T cells,the abnormal DCs function constitute a primary immune system defect associated with spontaneous development of lung inflammation in the KO mice[15].

Similar to the result of our previous study on rat model with IBS, the colonic level of Runx3 mRNA and TGF-β_1 mRNA did not show any remarkable changes in IBS group compared with their control group. This negative data may be result from the clinic type of IBS, the lasting time of the inflammation and the depth of the samples. But the expression of CD83 mRNA was higher than the control group, which indicates the maturation of dendritic cells maybe a important role in IBS patients.

Interestingly, in IBS patients, there is a significant correlation between Runx3 mRNA and TGF-β_1, suggesting that Runx3 - TGF-β_1 inflammation pathway might be involved in human IBS. Runx3 mRNA and CD83 mRNA also have certain correlation, but there is no correlation between TGF-β_1 mRNA and Runx3 mRNA,this may imply that Runx3 may regulate the maturation of dendritic cells through other signal transduction system in IBS patients.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Gwee KA, Bak YT, Ghoshal UC, Gonlachanvit S, Lee OY, Fock KM, et al. Asian consensus on irritable bowel syndrome. *J Gastroenterol Hepatol* 2010; **25**: 1189-1205.

[2] Lee HF, Hsieh JC, Lu CL, Yeh TC, Tu CH, Cheng CM, et al. Enhanced affect/cognition-related brain responses during visceral placebo analgesia in irritable bowel syndrome patients. *Pain* 2012; **153**: 1301-1310.

[3] Steinman RM. Dendritic cells: versatile controllers of the immune system. *Nat Med* 2007; **13**: 1155-1159.

[4] Steinman RM, Hemmi H. Dendritic cells: translating innate to adaptive immunity. *Curr Top Microbiol Immunol* 2006; **311**: 17-58.

[5] Sugai M, Aoki K, Osato M, Nambu Y, Ito K, Taketo MM, et al. Runx3 is required for full activation of regulatory T cells to prevent colitis-associated tumor formation. *J Immunol* 2011; **186**: 6515-6520.

[6] Kohu K, Kubo M, Ichikawa H, Ohno S, Habu S, Sato T, et al. Pleiotropic roles of Runx transcription factors in the differentiation and function of T lymphocytes. *Curr Immunol Rev* 2008; **4**: 101-115.

[7] Corinaldesi R, Stanghellini V, Cremon C, Gargano L, Cogliandro RF, De Giorgio R, et al. Effect of mesalazine on mucosal immune biomarkers in irritable bowel syndrome:a randomized controlled proof-of-concept study. *Aliment Pharmacol Ther* 2009; **30**: 245-252.

[8] Feng B, La JH, Schwartz ES, Gebhart GF. Neural and neuro-immune mechanisms of visceral hypersensitivity in irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G1085-G1098.

[9] Krajina T, Leithauser F, Moller P, Trobonjaca Z, Reimann J. Colonic lamina propria dendritic cells in mice with $CD4^+$ T cell-induced colitis. *Eur J Immunol* 2003; **33**: 1073- 1083.

[10] Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 cells. *Annu Rev Immunol* 2009; **27**: 485-517.

[11] Murakami H, Akbar SM, Matsui H, Horiike N, Onji M. Macrophage migration inhibitory factor activates antigen-presenting dendritic cells and induces inflammatory cytokines in ulcerative colitis. *Clin Exp Immunol* 2002; **128**: 504-510.

[12] Cremon C, Gargano L, Morselli-Labate AM, Santini D, Cogliandro RF, De Giorgio R. et al. Mucosal immune activation in irritable bowel syndrome: gender-dependence and association with digestive symptoms. *Am J Gastroenterol* 2009; **104**: 392-340.

[13] Fogel-Petrovic M, Long JA, Misso NL, Foster PS, Bhoola KD, Thompson PJ. Physiological concentrations of transforming growth factor beta1selectively inhibit human dendritic cell function. *Int Immunopharm* 2007; **7**: 1924-1933.

[14] Ohtani T, Mizuashi M, Nakagawa S, Sasaki Y, Fujimura T, Okuyama R, Aiba S. TGF-beta1 dampens the susceptibility of dendritic cells to environmental stimulation, leading to there quirement for danger signals for activation. *Immunol* 2009; **126**: 485-499.

[15] Fainaru O, Woolf E, Lotem J, Yarmus M, Brenner O, Goldenberg D, et al. Runx3 regulated mouse TGF-β -mediated dendritic cell function and its absence results in airway inflammation. *EMBO J* 2004; **23**: 969-979.