Journal of Hainan Medical University

http://www.hnykdxxb.com

SOCS2 and SOCS3 expression in ulcerative colitis and their correlation with inflammatory response and immune response

Le Huang[1], Xiao−Lin Sun[1], Peng−Fei Liu[2]✉

[1] *Department of Gastroenterology, Central Hospital of Zibo Mining Refco Group Ltd, Zibo, Shandong Province, 255120*

[2] *Department of Gastroenterology, Affiliated Hospital of Yan´an University, Yan´an, Shaanxi Province, 716000*

ARTICLE INFO

Article history:
Received 11 May 2017
Received in revised form 18 May 2017
Accepted 25 May 2017
Available online 24 Jun 2017

Keywords:
Ulcerative colitis
SOCS2
SOCS3
Inflammatory response
Immune response

ABSTRACT

Objective: To study the correlation of SOCS2 and SOCS3 expression in ulcerative colitis tissue with inflammatory response and immune response. **Methods:** Ulcerative colitis lesions and normal mucosa from colonoscopic biopsy in Central Hospital of Zibo Mining Refco Group Ltd between May 2014 and July 2016 were selected and enrolled in UC group and control group respectively. RNA was extracted to determine mRNA expression of SOCS2 and SOCS3 as well as inflammatory response JAKs/STATs pathway molecules; protein was extracted to determine the contents of immune response cytokines. **Results:** SOCS2 mRNA expression in intestinal mucosa of UC group was not significantly different from that of control group, and SOCS3 mRNA expression was significantly lower than that of control group; JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression as well as IFN-γ and IL-17 protein contents in intestinal mucosa of UC group were significantly higher than those of control group while IL-4 and IL-10 protein contents were significantly lower than those of control group; JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression as well as IFN-γ and IL-17 protein contents in UC group of intestinal mucosa with low SOCS3 expression were significantly higher than those of intestinal mucosa with high SOCS3 expression while IL-4 and IL-10 protein contents were significantly lower than those of intestinal mucosa with high SOCS3 expression. **Conclusion:** Low expression of SOCS3 in ulcerative colitis can aggravate the inflammatory reaction and cause the imbalance of Th1/Th2 and Th17/Treg immune response.

1. Introduction

Ulcerative colitis (UC) is a type of inflammatory bowel disease with unknown pathogenesis, which can involve the colon and rectum and cause abdominal pain, diarrhea and bloody mucopurulent stool. Abnormal inflammation and immune response disorder in intestinal mucosa are the important pathologic characteristics of ulcerative colitis[1,2], but the mechanisms regulating intestinal mucosal inflammation and immune response haven′t been clarified. Janus kinase (JAK) - signal transducer and activator of transcription (STAT) are the important pathways that regulate inflammation and immune response[3,4], and abnormal JAK-STAT pathway is closely related to the incidence of ulcerative colitis[5]. SOCS2 and SOCS3 in suppressors of cytokine signaling (SOCS) family are the JAK-STAT pathway inhibitors, and existing animal experimental study has confirmed that in rat model with ulcerative colitis induced by trinitrobenzene sulfonic acid, SOCS2 and SOCS3 expression obviously change and are associated with the increase of intestinal mucosal inflammation[6]. At present, the changes of SOCS2 and SOCS3 in ulcerative colitis lesions and their relationship with inflammatory response and immune response are not clear. In the following studies, the correlation of SOCS2 and SOCS3 expression in ulcerative colitis tissue with inflammatory response and immune response was analyzed.

✉Corresponding author: Peng-Fei Liu, Department of Gastroenterology, Affiliated Hospital of Yan'an University, Yan'an, Shaanxi Province, 716000.
Tel: 0533-5854574; 13864339033
Fund Project: Health Research Projects of Shaanxi Health and Family Planning Commission No: 2014D30.

2. Materials and methods

2.1 Clinical samples

Ulcerative colitis lesions from colonoscopic biopsy in Central Hospital of Zibo Mining Refco Group Ltd between May 2014 and July 2016 were selected as the UC group of the research, a total of 46 cases were included, and they were from 29 male cases and 17 female cases that were 43-59 years old; normal mucosa tissues from colonoscopic biopsy during the same period were selected as the control group of the research, a total of 60 cases were included, and they were from 36 male cases and 24 female cases that were 38-60 years old. There was no significant difference in the general data between the two groups of subjects (P>0.05).

2.2 Experimental materials

RNA extraction reagent Trizol was from Invitrogen Company, cDNA synthesis kit and PCR kit were from Promega Company, protein extraction reagent RIPA and BCA kit were from Shanghai Beyotime Company, and enzyme-linked immunosorbent assay kit was from Shanghai Westang Company.

2.3 Experimental methods

2.3.1 RNA expression detection methods

Intestinal mucosa of UC group and control group were taken and added in RNA extraction reagent Trizol to extract the total RNA, cDNA synthesis kit was used for reverse transcription from total RNA samples into cDNA, PCR reaction kit was used for cDNA sample amplification, SOCS2, SOCS3, JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 were amplified respectively, and the mRNA expression was calculated.

2.3.2 Protein content detection methods

Intestinal mucosa of UC group and control group were taken and added in protein extraction reagent RIPA to extract the total protein, enzyme-linked immunosorbent assay kit was used to determine IFN-γ, IL-4, IL-10 and IL-17 content, the BCA kit was used to determine total protein content, and the IFN-γ, IL-4, IL-10 and IL-17 protein expression per unit mass total protein were calculated.

2.4 Statistical methods

SPSS 19.0 software was used to input and analyze data, the median of SOCS3 mRNA expression in intestinal mucosa of UC group was calculated and used as the standard to divide the UC group into high expression group and low expression group, measurement data analysis between two groups was by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 SOCS2 and SOCS3 expression in ulcerative colitis tissue

SOCS2 and SOCS3 mRNA expression in intestinal mucosa of UC group and control group were as follows: SOCS2 and SOCS3 mRNA expression in intestinal mucosa of UC group were (0.98±0.12) and (0.35±0.08) respectively; SOCS2 and SOCS3 mRNA expression in intestinal mucosa of control group were (1.04±0.17) and (1.02±0.15) respectively. After t test, SOCS2 mRNA expression in intestinal mucosa of UC group was not significantly different from that of control group (P>0.05), and SOCS3 mRNA expression was significantly lower than that of control group (P<0.05).

3.2 Inflammation JAKs/STATs pathway molecule expression in ulcerative colitis tissue

Analysis of inflammation JAKs/STATs pathway molecules JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression in intestinal mucosa tissue of UC group and control group were as follows: JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression in intestinal mucosa of UC group were significantly higher than those of control group. Differences were statistically significant in JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression in intestinal mucosa tissue of UC group and control group (P<0.05), shown in Table 1.

Analysis of JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression in UC group of intestinal mucosa tissue with low and high SOCS3 expression were as follows: JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression in UC group of intestinal mucosa with low SOCS3 expression were significantly higher than those of intestinal mucosa with high SOCS3 expression. Differences were statistically significant in JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression in UC group of intestinal mucosa tissue with low and high SOCS3 expression (P<0.05), shown in Table 2.

Table 1.

JAKs/STATs pathway molecule expression in intestinal mucosa tissue of UC group and control group.

Groups	n	JAK1	JAK2	JAK3	STAT1	STAT3	STAT5
UC group	46	2.25±0.34	1.98±0.29	2.41±0.42	1.92±0.26	2.38±0.44	1.79±0.25
Control group	60	1.05±0.16	1.02±0.18	0.98±0.11	1.07±0.19	0.96±0.13	1.04±0.11
T		12.198	9.284	14.586	8.895	13.558	7.697
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 2.

JAKs/STATs pathway molecule expression in UC group of intestinal mucosa tissue with different SOCS3 expression.

SOCS3	n	JAK1	JAK2	JAK3	STAT1	STAT3	STAT5
Low expression	23	2.98±0.42	2.60±0.39	3.13±0.51	2.41±0.34	3.11±0.55	2.31±0.36
High expression	23	1.52±0.23	1.39±0.22	1.67±0.29	1.47±0.20	1.54±0.18	1.35±0.18
T		9.181	8.988	8.281	7.484	10.495	8.752
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

3.3 Immune response cytokine contents in ulcerative colitis tissue

Analysis of immune response cytokines IFN-γ (ng/mg protein), IL-4 (pg/mg protein), IL-10 (pg/mg protein) and IL-17 (ng/mg protein) in intestinal mucosa tissue of UC group and control group were as follows: IFN-γ and IL-17 protein contents in intestinal mucosa of UC group were significantly higher than those of control group while IL-4 and IL-10 protein contents were significantly lower than those of control group. Differences were statistically significant in IFN-γ, IL-4, IL-10 and IL-17 protein contents in intestinal mucosa tissue of UC group and control group (P<0.05), shown in Table 3.

Analysis of IFN-γ, IL-4, IL-10 and IL-17 contents in UC group of intestinal mucosa tissue with low and high SOCS3 expression were as follows: IFN-γ and IL-17 protein contents in UC group of intestinal mucosa with low SOCS3 expression were significantly higher than those of intestinal mucosa with high SOCS3 expression while IL-4 and IL-10 protein contents were significantly lower than those of intestinal mucosa with high SOCS3 expression. Differences were statistically significant in IFN-γ, IL-4, IL-10 and IL-17 protein contents in UC group of intestinal mucosa tissue with low and high SOCS3 expression (P<0.05), shown in Table 4.

Table 3.

Immune response cytokine expression in intestinal mucosa tissue of UC group and control group.

Groups	*n*	IFN-γ	IL-4	IL-10	IL-17
UC group	46	5.85±0.77	132.57±17.96	85.56±10.12	2.95±0.52
Control group	60	2.45±0.41	332.48±54.82	221.35±30.28	1.24±0.18
T		12.585	15.492	16.028	11.952
P		<0.05	<0.05	<0.05	<0.05

Table 4.

Immune response cytokine expression in UC group of intestinal mucosa tissue with different SOCS3 expression.

SOCS3	*n*	IFN-γ	IL-4	IL-10	IL-17
Low expression	23	7.21±0.94	93.41±11.24	55.42±7.81	4.16±0.64
High expression	23	4.31±0.55	178.45±23.12	121.24±15.86	1.70±0.29
T		8.472	9.575	12.576	14.218
P		<0.05	<0.05	<0.05	<0.05

4. Discussion

The pathogenesis of ulcerative colitis is unknown so far, the abnormal inflammatory response and immune response disorder in intestinal mucosa are the important pathological characteristics of the disease[7], and the abnormal activation of JAK-STAT signaling pathways is associated with the abnormal expression and secretion of a variety of active cytokines during inflammatory reaction and immune response[8,9]. SOCS family includes eight members of SOCS1-7 and CIS1, SOCS2 and SOCS3 have been confirmed to be associated with the negative regulation of JAK-STAT signal pathway, and the changes of SOCS2 and SOCS3 expression will cause the abnormal activation of JAK-STAT signal pathway, and then result in the occurrence of a variety of inflammatory diseases and autoimmune diseases[10,11]. In the study, the analysis of SOCS2 and SOCS3 in the intestinal mucosa of ulcerative colitis and normal intestinal mucosa showed that SOCS2 mRNA expression in intestinal mucosa of UC group was not significantly different from that of control group, and SOCS3 mRNA expression was significantly lower than that of control group. This means that abnormal expression of SOCS3 may be associated with the incidence of ulcerative colitis, whereas SOCS2 does not play the decisive role in the development and change of in ulcerative colitis; lower expression of SOCS3 will weaken the inhibiting effect on downstream JAK-STAT signaling pathways, and then cause inflammatory reaction activation and immune response disorder through the abnormal activation of JAK-STAT signaling pathways.

In the JAK-STAT signaling pathway, the main molecules that regulate inflammation include the JAK1, JAK2 and JAK3 in the JAK family as well as the STAT1, STAT3 and STAT5 in the STAT family. The activated JAK1, JAK2 and JAK3 can cause STAT1, STAT3 and STAT5 gathering and activation within the cells, and then transfer from the cytoplasminto the nucleus to regulate the secretion of various cytokines and affect the inflammatory and immune response[12–14]. In the study, the analysis of JAK-STAT signal pathway molecule expression in the intestinal mucosa of ulcerative colitis and normal intestinal mucosa showed that JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression in intestinal mucosa of UC group were significantly higher than those of control group. This indicates that the abnormal activation of the JAK-STAT signaling pathway is associated with the occurrence of ulcerative colitis. Further analysis of the relationship between abnormal SOCS3 expression and abnormal JAK-STAT signal pathway activation showed that JAK1, JAK2, JAK3, STAT1, STAT3 and STAT5 mRNA expression in intestinal mucosa of ulcerative colitis with low SOCS3 expression were significantly higher than those of intestinal mucosa with high SOCS3 expression. This shows that the low expression of SOCS3 can facilitate the abnormal activation of the JAK-STAT signaling pathway in ulcerative colitis lesions.

The cytokine secretion regulated by the JAK-STAT signaling pathway is very important for maintaining the immune balance of Th1/Th2 and Th17/Treg in the CD4+T cell subsets. In the development and change of ulcerative colitis, the number and function of Th1/Th2 and Th17/Treg are in disorder, kinds, and the synthesis and secretion of a variety of cytokines are abnomral, which affect the abnormal inflammatory response and the immune

response disorder[15,16]. Th1 and Th2 are the first discovered CD4+T cell subsets, the IFN-γ, IL-2 and TNF-α secreted by the former can mediate cellular immune response and cause inflammatory tissue damage, the IL-4 and IL-5 secreted by the latter can mediate humoral immunity and restrain inflammation[17]; Th17/Tre is the newly discovered CD4+T cell subset, the IL-17 secreted by the former is an important pro-inflammatory factor, and the IL-10 secreted by the latter is an important inhibitory cytokine[18]. In the study, the analysis of Th1/Th2 and Th17/Treg cytokine expression in the intestinal mucosa of ulcerative colitis and normal intestinal mucosa showed that IFN-γ and IL-17 protein contents in intestinal mucosa of UC group were significantly higher than those of control group while IL-4 and IL-10 protein contents were significantly lower than those of control group. This indicates that the increased function of Th1 and Th17 and the decreased function of Th2 and Treg are related to the occurrence of ulcerative colitis. Further analysis of the relationship of abnormal SOCS3 expression with Th1/Th2 and Th17/Treg cytokine expression showed that IFN-γ and IL-17 protein contents in intestinal mucosa of ulcerative colitis with low SOCS3 expression were significantly higher than those of intestinal mucosa with high SOCS3 expression while IL-4 and IL-10 protein contents were significantly lower than those of intestinal mucosa with high SOCS3 expression. This shows that the low expression of SOCS3 can result in Th1/Th2 and Th17/Treg disorder, promote the activation of Th1 and Th17, and inhibit the activation of Th2 and Treg.

To sum up, it is believed that the low expression of SOCS3 is closely related to the occurrence and development of ulcerative colitis; the reduction of SOCS3 expression in the intestinal mucosa can cause the abnormal activation of JAK-STAT signaling pathway, and thus lead to the imbalance of the Th1/Th2 and Th17/Treg immune responses.

References

[1] Soufli I, Toumi R, Rafa H, Touil-Boukoffa C. Overview of cytokines and nitric oxide involvement in immuno-pathogenesis of inflammatory bowel diseases. *World J Gastrointest Pharmacol Ther* 2016; **7**(3): 353-360.

[2] Bjarnason I, Hayee B, Pavlidis P, Kvasnovsky C, Scalori A, Sisson G, et al. Contrasting pattern of chronic inflammatory bowel disease in primary and autoimmune sclerosing cholangitis. *EBio Medicine* 2015; **2**(10): 1523-1527.

[3] Roskoski R Jr. Janus kinase (JAK) inhibitors in the treatment of inflammatory and neoplastic diseases. *Pharmacol Res* 2016; **111**: 784-803.

[4] Galien R. Janus kinases in inflammatory bowel disease: Four kinases for multiple purposes. *Pharmacol Rep* 2016; **68**(4): 789-796.

[5] Zundler S, Neurath MF. Integrating immunologic signaling networks: the jak/stat pathway in colitis and colitis-associated cancer. *Vaccines (Basel)* 2016; **4**(1). E5.

[6] Zhou Tingting, Tong Qiaoyun, Yuan Jinhua. Expressions of SOCS2 and SOCS3 in colonic mucosa of rats with ulcerative colitis. *Acta Medicinae Universitatis Scientiae et Technologiae Huazhong* 2015; **44**(3): 285-288, 297.

[7] Grevenitis P, Thomas A, Lodhia N. Medical therapy for inflammatory bowel disease. *Surg Clin North Am* 2015; **95**(6): 1159-1182.

[8] Villarino AV, Kanno Y, O'Shea JJ. Mechanisms and consequences of Jak-STAT signaling in the immune system. *Nat Immunol*, 2017, **18**(4):374-384.

[9] Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM. JAK-STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects. *Drugs* 2017; **77**(5): 521-546.

[10]Liu Z, Gan L, Zhou Z, Jin W, Sun C. SOCS3 promotes inflammation and apoptosis via inhibiting JAK2/STAT3 signaling pathway in 3T3-L1 adipocyte. *Immunobiology* 2015; **220**(8): 947-953.

[11]Rupp R, Senin P, Sarry J, Allain C, Tasca C, Ligat L, et al. A point mutation in suppressor of cytokine signalling 2 (socs2) increases the susceptibility to inflammation of the mammary gland while associated with higher body weight and size and higher milk production in a sheep model. *PLoS Genet* 2015; **11**(12): e1005629.

[12]Hedl M, Proctor DD, Abraham C. JAK2 disease-risk variants are gain of function and JAK signaling threshold determines innate receptor-induced proinflammatory cytokine secretion in macrophages. *J Immunol* 2016; **197**(9): 3695-3704.

[13]Can G, Tezel A, Gurkan H, Tozkır H, Unsal G, Soylu AR, et al. Investigation of IL23R, JAK2, and STAT3 gene polymorphisms and gene-gene interactions in Crohn's disease and ulcerative colitis in a Turkish population. *Turk J Gastroenterol* 2016; **27**(6): 525-536.

[14]Mariman R, Tielen F, Koning F, Nagelkerken L. The probiotic mixture VSL#3 dampens LPS-induced chemokine expression in human dendritic cells by inhibition of STAT-1 phosphorylation. *PLoS One* 2014; **9**(12): e115676.

[15]Fonseca-Camarillo G, Yamamoto-Furusho JK. Immunoregulatory pathways involved in inflammatory bowel disease. *Inflamm Bowel Dis* 2015; **21**(9): 2188-2193.

[16]Silva FA, Rodrigues BL, Ayrizono ML, Leal RF. The immunological basis of inflammatory bowel disease. *Gastroenterol Res Pract* 2016; **2016**: 2097274

[17]Heilmann RM, Suchodolski JS. Is inflammatory bowel disease in dogs and cats associated with a Th1 or Th2 polarization. *Vet Immunol Immunopathol* 2015; **168**(3-4): 13113-13114.

[18]Kryczek I, Wang L, Wu K, Li W, Zhao E, Cui T, et al. Inflammatory regulatory T cells in the microenvironments of ulcerative colitis and colon carcinoma. *Oncoimmunology* 2016; **5**(8): e1105430.