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Original Article

Aberrant Promoter Methylation of the *Suppressor of Cytokine Signaling 3* (*SOCS3*) Gene in Colorectal Mucosa Is Associated with Susceptibility to Ulcerative Colitis

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Abstract

Background: Growing evidence supports that changes in the expression of the *suppressor of cytokine signaling 3* (SOCS3) protein contribute to the pathogenesis of types of inflammatory bowel diseases (IBDs), including ulcerative colitis (UC). Despite the importance of the currently known genetic risk map, an increasing number of observations reveal that epigenetic modifications, including DNA methylation, are considered as or even more important for IBD pathogenesis than genetic predisposition. We investigated the hypothesis that alterations in DNA methylation status at the promoter region within the *SOCS3* gene in colorectal tissue specimens may be involved in the susceptibility to UC.

Methods: We studied extracted DNA from colorectal biopsies of 15 ulcerative colitis cases and 15 age—and sex-matched healthy controls and performed genotype analyses of the promoter methylation status of the *SOCS3* gene, using the real-time quantitative multiplex methylation-specific PCR (QM-MSP) assay to show evidence of differential methylation between cases of ulcerative colitis and healthy controls.

Results: Based on methylation assay data profiling, we found that the mean CpG island methylation levels at the *SOCS3* gene promoter region in colorectal mucosa of patients with UC was significantly higher than mucosa from healthy controls (ulcerative colitis vs. healthy controls; 0.00007±0.0018 vs. 0.07±0.142, $p<0.000$).

Conclusion: Our data provide an important insight into the influence of epigenetic aberrances in the *SOCS3* gene such that the inactivation of the *SOCS3* gene by promoter hypermethylation might be a risk factor for inflamed mucosa of UC. It might also fundamentally contribute to the initiation of the inflammatory process and development of UC.

Keywords: DNA Methylation; Epigenetics; Immune Regulation; SOCS3; Ulcerative Colitis

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Introduction

Inflammatory bowel disease (IBD) is mainly characterized by chronic recurrent intestinal inflammation of all or part of the digestive tract with unclear etiology, which includes Crohn's disease (CD) and ulcerative colitis (UC) (1, 2). UC is a major manifestation of IBD and is histopathologically characterized by an even and continuous distribution of an inflammation that affects the intestinal mucosa ascending from the rectum into the colon (3, 4). The luminal microflora, the intestinal mucosa, and the host immunoregulatory factors are among the main biological networks involved in the development of IBD (3, 5, 6).

Host genetics and environmental triggers tightly modulate the interactions among these biological systems and influence predilection toward IBD (1, 5). Over the past few decades, major advances using new high-throughput sequencing methods have occurred in understanding with regard to genetic susceptibility to the disease (5, 7). However, these observations raise major questions supporting non-genetic alterations being as or even more important for IBD pathogenesis than genetic predisposition. In UC pathogenesis, the molecular mechanisms and the key biological components that such factors may influence are also partially understood (5, 8).

Epigenetics is a steadily growing research area in many human diseases, especially in inflammatory autoimmune diseases, including UC. Epigenetic changes are sensitive non-genetic alterations that are affected by a number of environmental factors (5, 9). Epigenetic pathways modulate gene expression without changing the DNA sequence (5, 8). Among epigenetic changes, DNA methylation (methylation of cytosines in CpG dinucleotides) is a fundamental mechanism of the epigenetic long-term modulation system of gene expression in mammals in which this epigenetic information can be transmitted through repetitive cell divisions and subsequently during the lifetime of an individual (5, 10-12).

Our current understanding of UC pathogenesis is incomplete but suggests that the disease develops as a consequence of a dysregulated immune response to the intestinal microbiota in genetically susceptible individuals, indicating the intercalating relationships among the main biological systems involved in gastrointestinal disorders. (7). It is hypothesized that environmental triggers during specific periods of development may induce critical modifications in an isolated or similar manner within the intestinal biological networks and lead to increased susceptibility to IBD (5).

Recent studies have also revealed novel functions for epigenetic alterations in diverse processes, such as regulation of the immune system, and have been implicated in the pathogenesis of some immune-mediated diseases, including IBD (5, 13). Several studies performed in UC demonstrate that among the many innate and adaptive immune pathways implicated, the regulation of immune cells and signaling pathways, including signaling pathway regulation components such as JAK-STAT signaling, are also involved.

JAK-STAT pathways are involved in cell-fate decisions in many mechanisms of immune cell functions. The major transcription factors, including STAT3, have been demonstrated to play a crucial role in transmitting inflammatory cytokine (IL-6, TNF-α, and IFN-γ) signals to the nucleus (3, 14). STAT pathways are also under classical feedback control through SOCS proteins, a family of intracellular proteins that are induced by STATs in response to cytokine stimulation (14). The *suppressor of cytokine signaling 3* (SOCS3) protein attenuates or terminates the activation of STAT3 by targeting the receptor and its associated JAK kinases for degradation (14). It has been reported that SOCS3 is significantly lower in the colonic tissue of patients with UC (3). However, assessment of DNA methylation changes of the *SOCS3* gene has rarely been addressed regarding UC susceptibility.

Because SOCS3 is involved in IBDs, and DNA methylation is important in regulating gene expression, we investigated the hypothesis that alterations in CpG island methylation status at the *SOCS3* gene promoter region in colorectal tissue specimens may be involved in susceptibility to UC.

Materials and Methods

Patients and Tissue Samples

All colorectal mucosa specimens analyzed in this retrospective study were obtained from colonoscopic mucosal resection at the gastroen-

terology clinics of Kasra and Laleh hospitals in Tehran, Iran, between May 2014 and July 2015. Promoter methylation of the *SOCS3* gene was examined in colorectal mucosal specimens of all 15 UC patients (8 females, seven males) and 15 age- and sex-matched healthy controls (8 females, 7 males).

The histopathological diagnosis was confirmed by H&E-stained sections blinded to all clinical characteristics. Colonic mucosal biopsy specimens of healthy controls were taken at routine check-ups for colonoscopies performed on patients who had no history of IBD or other gastrointestinal abnormalities. Furthermore, healthy subjects took no regular medications and had normal results on the routine laboratory tests.

Ethical Statement

The Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran, approved this study's tissue collection and protocol. Written consent was obtained from all participants.

DNA Preparation and Bisulfite Conversion System

According to the manufacturer's instructions, the genomic DNA of colonic mucosa was isolated from each subject using the High Pure PCR Template Preparation Kit (Roche). Bisulfite modification of genomic DNA was done using the commercially available MethylEdge™ Bisulfite Conversion System (Promega, Madison, WI) as per the manufacturer's instructions. After treatment with sodium bisulfite, unmethylated cytosine residues are converted to uracil, whereas 5-methylcytosine (5mC) remains unaffected. After PCR amplification, uracil residues are converted to thymine. Bisulfite-modified DNA specimens were aliquoted and stored at -20°C.

Methylation Analysis

Genomic DNA specimens were evaluated to determine the methylation status of CpG sites across the promoter region of the *SOCS3* gene using a SYBR green dye-based DNA methylation assay named the real-time quantitative multiplex methylation-specific PCR (QM-MSP) method (15). The MethySYBR procedure, which is highly sensitive and highly specific, requires two sequential steps of PCR reactions. The first pre-amplification PCR reaction (the multiplex step) was carried out with MethySYBR primers, including external forward primer (EXT-F; 5'-GTAGGGAGGTGACGAGGTAGG-3') and external reverse primer (EXT-R; 5'-ACAAAATA-ACCCCGAACAACC-3'). The primer set was designed to enable the simultaneous amplification of many discrete target alleles in a single reaction. The PCR reaction was performed in a volume of 25 µl containing 1 µl of converted genomic DNA. The DNA was denatured at 95C for 5 min, followed by 30 cycles at 94C for 30 s, 56C for 30 s, and 72C for 30 s, with a final extension at 72C for 5 min.

In the second round of PCR, the specific methylated target is quantified from multiplex step products using both nested methylation-independent and methylation-specific primer sets, including nested methylation-specific forward (FM; 5'-GGAGATTTTAGGTTTTCGGAATATTTC-3') and reverse primer (RM; 5'- CCCCCGAAAC-TACCTAAACGCCG-3'). The methylation profile of the promoter CpG islands was defined based on the UCSC database. A methylation-specific primer design for the *SOCS3* gene was adopted from the group of Li *et al.* (4). Also, CpG island prediction and primer blasting were performed using the MethBlast tool. The bisulfite-treated DNA was PCR amplified in a 10 µl reaction volume containing 5 µl SYBR® Green Master Mix, 0.25 µl of each of the methylated primers, 3.5 µl DDW, and 1 µl of bisulfite-treated DNA. Cycling conditions were: 1 min at 95°C, 30 cycles of 30 s at 94°C, 1 min at 60°C, 30 s at 72°C subsequently followed by 5 min at 72°C.

Quantitative MSP was performed with a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). No untreated template controls were included in each run as negative controls. Fully converted methylated human plasmid DNA was used as a positive control for MSP in each run to serve as the 100% methylated reference for calculating the relative methylation percentages of DNA samples.

In order to calculate the ratio of unmethylated versus total amplifiable bisulfite-treated DNA, the $\Delta\Delta C_q$ method was used as the cycle of quantification (C_q) for the reaction between methylation-specific primers (MSP) and bisulfite-specific primers (BSP) was obtained (16, 17). Using a

reference sample for standardization indicates the relative difference between the template of interest and a control template:

Unmethylated DNA level $2^{-\Delta\Delta CT}$

$\Delta\Delta C_q = \Delta C_{q\ sample} - \Delta C_{q\ plasmid}$

$\Delta C_{q\ sample} = C_{q\ MCP} - C_{q\ BSP}$

$\Delta C_{q\ plasmid} = C_{q\ MCP} - C_{q\ BSP}$

All cycle threshold (CT) values were obtained in the exponential phase and normalized by subtraction of the CT value. The fold change in target gene samples, after normalization with the expression of PCR products amplified by external nested primers as internal control (BSP), was calculated using the $2^{-\Delta\Delta CT}$ method, where ΔΔCT=ΔCT (samples) - ΔCT (controls) and ΔCT was calculated by transforming the difference in CT values of target gene vs. the BSP products.

Statistical Analysis

DNA methylation data were expressed as mean ± SD. The difference in the mean promoter methylation levels between the two groups was compared using the Mann-Whitney test. The chi-square statistic was used to discover if there is a relationship between two categorical variables. The odds ratio (OR) and 95% confidence interval were considered to calculate the measure of association. All tests were 2-sided, and the level of significance was set at $P<0.05$. All statistical calculations were made using the SPSS 21.0 version.

Results

Because some genes are shown to be methylated with aging (18, 19) and sex (20), age- and sex-matched healthy controls were studied. The study consisted of 15 UC patients, with a mean age of 45±15.42 years (range: 25–82 years), and included 7 (46.7%) males and 8 (53.3%) females. Furthermore, fifteen were healthy controls (8 females, 7 males), with a mean age of 46±15.38 years (range: 28–78 years).

The use of SYBR green dye during quantitative PCR enables melting curve analysis of target amplicons to determine the methylation status of CpG sites on the *SOCS3* gene. The resulting melting curve for the *SOCS3* gene is shown in **Figure 1**.

The promoter methylation status of the *SOCS3* gene in colonic mucosa specimens of all 15 UC patients and 15 paired healthy controls were compared **(Figure 2)**. Methylation assay data profiling showed evidence of differential promoter methylation levels between patients with UC and healthy controls in which the mean methylation levels of the *SOCS3* gene was significantly higher in the mucosa of UC (0.00007±0.0018) than in mucosa of healthy controls (0.07±0.142) ($p<0.000$, **Figure 2**).

Qualitative methylation data analysis of each individual showed a significant difference in the frequency of promoter methylation status between rectal inflammatory mucosa (11/15, 73.3%) and paired normal terminal ileum (2/15, 13.3%). The relative risk of disease indicated that individuals who had an aberrant promoter methylation profiling were at high risk of the development of UC ($p=0.003$, OR= 17.88, CI=2.15-196.97).

Discussion

The exact cause of IBD remains unknown and is partially explained by the currently known genetic risk map. There is convincing evidence of interactions between genetic and epigenetic regulation in the human genome on gene regulation in which environmentally sensitive non-genetic alterations modulate the epigenetic modifications. It is hypothesized that UC could be considered a disorder where epigenetic dysregulation may play an important etiologic role (10, 12).

In the present study, we investigated the DNA methylation status of *SOCS3* promoter region in UC patients. Our findings showed that the hypermethylation in the *SOCS3* gene in the rectal mucosa of UC patients is associated with a higher risk of developing UC.

Interestingly, when analyzing the methylation status of the *SOCS3* gene, we observed very low levels of unmethylated DNA in both groups. In suppression of cytokine-induced signaling, the maximum inhibitory activity of SOCS, including SOCS3 protein, toward normal STAT signaling can be achieved by very low levels of SOCS expression (21).

Epigenome-Wide Association Studies (EWAS) of DNA methylation using peripheral blood-derived DNA from IBD patients revealed differential methylation levels of fifty genes in IBD patients than the control group, including some involved in immune system activation (22). Recent studies using evidence from the high rate of discordance

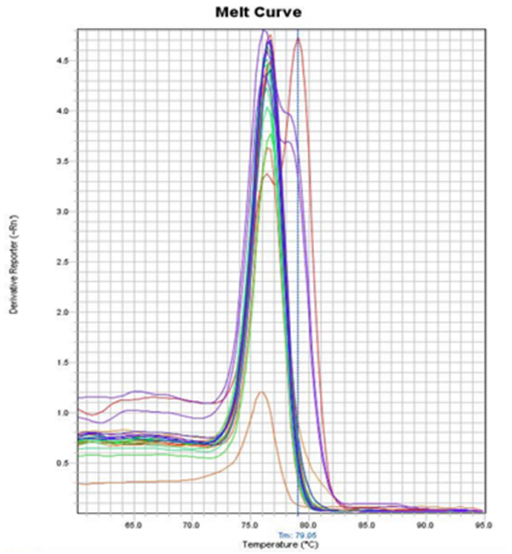

Figure 1. The figure shows the fluorescent melting peaks for the promoter CpG island of the *SOCS3* gene. DNA melt curve analysis of changes in CpG methylation status was analyzed based on amplification of unmethylated bisulfite-treated DNA from colorectal biopsies of UC cases.

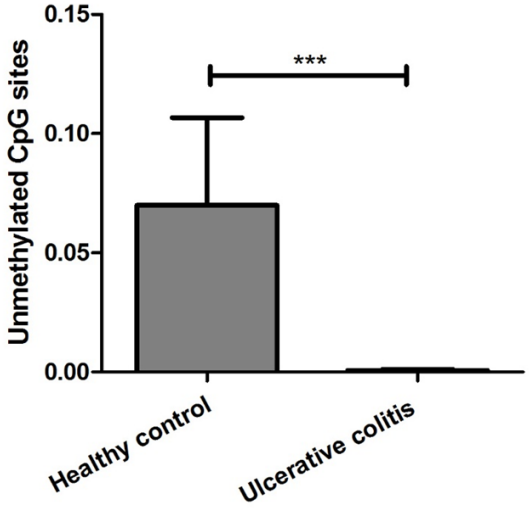

Figure 2. Comparison of changes in CpG island methylation status at the *SOCS3* gene promoter region in colorectal tissue specimens of patients with ulcerative colitis and healthy control. Error bars mean ± SD (*$p < 0.05$).

in monozygotic twins and a sharp increase in the incidence of IBD implicate environmental factors as major and key play even than genetic predisposition (17). Epigenetic alternations of some immunoregulatory molecules have already been described in colorectal specimens of patients with UC (23).

Increased SOCS3 expression has been identified in mouse models of IBD, which in turn leads to limiting the extent of inflammation (24). On the other hand, it has been proposed that concomitant with the loss of SOCS3 expression, STAT3 activation increased, providing a rational mechanism to explain why the loss of SOCS3 expression is linked to an increased predilection towards the development of certain inflammatory autoimmune diseases such as UC (4).

Previously, it has been reported that the *SOCS3* promoter methylation is a major regulator of SOCS3 expression in the colon epithelial cells (4). Hypermethylation of CpG islands of the *SOCS3* gene causing loss of negative feedback loop of

STAT3 activation and role of STAT3/SOCS3 dysregulation has also been frequently observed in various human immunological disorders, including neuroinflammatory diseases (14) and a variety of cancers (25-28).

Reduced levels of SOCS3 expression might be involved in the development and progression of cancer, suggesting an important anti-tumor activity of SOCS3 downregulation (4). Inflammatory bowel disease-associated colorectal cancer (IBD-CRC) often exhibits CpG hypermethylation of the *SOCS3* gene, which inactivation of the gene in turn provokes hyperactivity of the inflammation-related IL6-STAT3 signaling cascade and consequent tumor initiation. Mouse models have shown that IL-6 produced via cells in the gut lamina propria and subsequent STAT3 activation are required for the proliferation of tumor-initiating cells and survival of premalignant intestinal epithelial cells (4, 29).

IL-6 signaling has also been reported to provoke an increase in methylation of *SOCS3* by stimulating increased DNA methyltransferase expression via suppression of DNA methyltransferase-targeting microRNAs, and that results in increased signaling through STAT3 (4, 30).

SOCS3, a negative suppressor of STAT3 that is itself regulated by promoter hypermethylation, provides an additional regulation to this transcription factor (31). The way these alterations in DNA methylation of important cell regulators such as SOCS3 cooperate in an unabated mucosal inflammatory process is not yet clear (4). Loss of epithelial SOCS3 expression is related to *SOCS3* gene promoter methylation and may involve reduced negative suppression of IL-6 signaling toward STAT3 activation (4).

Here, we have found that hypermethylation of CpG islands of the *SOCS3* gene might cause loss of negative feedback loop of STAT3 signaling and role of STAT3/SOCS3 signaling pathway dysregulation. The altered DNA methylation profile of the *SOCS3* gene can result in unabated stimulation of the STAT3 pathway. Consequently, the transcription factor STAT3, which is activated during inflammation, triggers the process of inflammation (31) and provides the loss of mucosal immune tolerance and uncontrolled responses to microbial antigens (32).

It has been reported that methylation of some promoter CpG islands does not interfere with gene expression. Although epigenetic differences between patients and controls are identified, it is also possible that SOCS3 methylation may not be related to *SOCS3* gene expression. Hence, the expression pattern of the *SOCS3* gene should be investigated along with the DNA methylation status of the *SOCS3* promoter region.

In summary, we did a case-control association study and detected new profiles of CpG hypermethylation of the *SOCS3* gene that were associated with the susceptibility to UC development. We report that the hypermethylation status of SOCS3 in colorectal tissue specimens of UC patients is significantly higher compared to healthy controls. Our findings suggest that the CpG hypermethylation at the promoter region of a gene might influence *SOCS3* gene expression and are fundamentally related to the etiology of UC. Our data provide an important insight into the STAT/SOCS axis by representing that epigenetic modifications in the *SOCS3* gene regulation are the basis for hyperactivity of the inflammation-related IL6-STAT3 in UC patients. Further studies using in vivo and ex vivo systems will be needed to confirm our findings and even propose that aberrant methylation could be considered as a potential target for the rational treatment of UC.

Conflict of Interest

All the authors approved that they have no conflict of interest. Statement of Author Contributions

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References

1. Kim SW, Kim ES, Moon CM, Park JJ, Kim TI, Kim WH, et al. Genetic polymorphisms of IL-23R and IL-17A and novel insights into their associations with inflammatory bowel disease. Gut. 2011;60(11):1527-36.
2. Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun. 2000;68(12):7010-17.
3. Li Y, de Haar C, Peppelenbosch MP, van der

Woude CJ. SOCS3 in immune regulation of inflammatory bowel disease and inflammatory bowel disease-related cancer. Cytokine Growth Factor Rev. 2012;23(3):127-38.
4. Li Y, Deuring J, Peppelenbosch MP, Kuipers EJ, de Haar C, van der Woude CJ. IL-6-induced DNMT1 activity mediates SOCS3 promoter hypermethylation in ulcerative colitis-related colorectal cancer. Carcinogenesis. 2012:bgs214.
5. Kellermayer R. Epigenetics and the developmental origins of inflammatory bowel diseases. Can J Gastroenterol. 2012;26(12):909.
6. Nakahara S, Arimura Y, Saito K, Goto A, Motoya S, Shinomura Y, et al. Association of SLC22A4/5 polymorphisms with steroid responsiveness of inflammatory bowel disease in Japan. Dis Colon Rectum. 2008;51(5):598-603.
7. Nimmo ER, Prendergast JG, Aldhous MC, Kennedy NA, Henderson P, Drummond HE, et al. Genome-wide methylation profiling in Crohn's disease identifies altered epigenetic regulation of key host defense mechanisms, including the Th17 pathway. Inflamm Bowel Dis. 2012;18(5):889-99.
8. Kellermayer R, Dowd SE, Harris RA, Balasa A, Schaible TD, Wolcott RD, et al. Colonic mucosal DNA methylation, immune response, and microbiome patterns in Toll-like receptor 2-knockout mice. FASEB J. 2011;25(5):1449-60.
9. Tahara T, Arisawa T. Potential usefulness of DNA methylation as a risk marker for digestive cancer associated with inflammation. Expert Rev Mol Diagn. 2012;12(5):489-97.
10. Balasa A, Gathungu G, Kisfali P, Smith EOB, Cho JH, Melegh B, et al. Assessment of DNA methylation at the interferon regulatory factor 5 (IRF5) promoter region in inflammatory bowel diseases. Int J Colorectal Dis. 2010;25(5):553-56.
11. Tahara T, Shibata T, Nakamura M, Yamashita H, Yoshioka D, Okubo M, et al. Promoter methylation of protease-activated receptor (PAR2) is associated with severe clinical phenotypes of ulcerative colitis (UC). Clin Exp Med. 2009;9(2):125-30.
12. Häsler R, Feng Z, Bäckdahl L, Spehlmann ME, Franke A, Teschendorff A, et al. A functional methylome map of ulcerative colitis. Genome Res. 2012;22(11):2130-37.
13. Cooke J, Zhang H, Greger L, Silva AL, Massey D, Dawson C, et al. Mucosal genome-wide methylation changes in inflammatory bowel disease. Inflamm Bowel Dis. 2012;18(11):2128-37.
14. Egwuagu CE. STAT3 in CD4+ T helper cell differentiation and inflammatory diseases. Cytokine. 2009;47(3):149-56.
15. Lo P-K, Watanabe H, Cheng P-C, Teo WW, Liang X, Argani P, et al. MethySYBR, a novel quantitative PCR assay for the dual analysis of DNA methylation and CpG methylation density. J Mol Diagn. 2009;11(5):400-14.
16. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nat Protoc. 2008;3(6):1101-08.
17. Husseiny MI, Kuroda A, Kaye AN, Nair I, Kandeel F, Ferreri K. Development of a quantitative methylation-specific polymerase chain reaction method for monitoring beta cell death in type 1 diabetes. PLoS One. 2012;7(10):e47942.
18. Issa J-PJ, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet. 1994;7(4):536-40.
19. Ahuja N, Li Q, Mohan AL, Baylin SB, Issa J-PJ. Aging and DNA methylation in colorectal mucosa and cancer. Cancer Res. 1998;58(23):5489-94.
20. Liu J, Morgan M, Hutchison K, Calhoun VD. A study of the influence of sex on genome wide methylation. PLoS One. 2010;5(4):e10028.
21. Song MM, Shuai K. The suppressor of cytokine signaling (SOCS) 1 and SOCS3 but not SOCS2 proteins inhibit interferon-mediated antiviral and antiproliferative activities. J Biol Chem. 1998;273(52):35056-62.
22. Ventham NT, Kennedy NA, Nimmo ER, Satsangi J. Beyond gene discovery in inflammatory bowel disease: the emerging role of epigenetics. Gastroenterology. 2013;145(2):293-308.
23. Azarschab P, Porschen R, Gregor M, Blin N, Holzmann K. Epigenetic control of the E-cadherin gene (CDH1) by CpG methylation in colectomy samples of patients with ulcerative colitis. Genes Chromosomes Cancer. 2002;35(2):121-26.
24. Bluyssen HA, Rastmanesh MM, Tilburgs C, Jie K, Wesseling S, Goumans M-J, et al. IFNγ-dependent SOCS3 expression inhibits IL-6-induced STAT3 phosphorylation and differentially affects IL-6 mediated transcriptional responses in endothelial cells. Am J Physiol Cell Physiol. 2010;299(2):C354-C62.
25. He B, You L, Uematsu K, Zang K, Xu Z, Lee AY, et al. SOCS-3 is frequently silenced by hypermethylation and suppresses cell growth in human lung cancer. Proc Natl Acad Sci U S A. 2003;100(24):14133-38.
26. Sutherland KD, Lindeman GJ, Choong DY, Wittlin S, Brentzell L, Phillips W, et al. Differential hypermethylation of SOCS genes in ovarian and breast carcinomas. Oncogene. 2004;23(46):7726-33.

27. Weber A, Hengge UR, Bardenheuer W, Tischoff I, Sommerer F, Markwarth A, et al. SOCS-3 is frequently methylated in head and neck squamous cell carcinoma and its precursor lesions and causes growth inhibition. Oncogene. 2005;24(44):6699-708.
28. Niwa Y, Kanda H, Shikauchi Y, Saiura A, Matsubara K, Kitagawa T, et al. Methylation silencing of SOCS-3 promotes cell growth and migration by enhancing JAK/STAT and FAK signalings in human hepatocellular carcinoma. Oncogene. 2005;24(42):6406-17.
29. Li Y, de Haar C, Chen M, Deuring J, Gerrits MM, Smits R, et al. Disease-related expression of the IL6/STAT3/SOCS3 signalling pathway in ulcerative colitis and ulcerative colitis-related carcinogenesis. Gut. 2010;59(2):227-35.
30. Olaru AV, Cheng Y, Agarwal R, Yang J, David S, Abraham JM, et al. Unique patterns of CpG island methylation in inflammatory bowel disease-associated colorectal cancers. Inflamm Bowel Dis. 2012;18(4):641-48.
31. Hartnett L, Egan LJ. Inflammation, DNA methylation and colitis-associated cancer. Carcinogenesis. 2012:bgs006.
32. Fu XY. STAT3 in immune responses and inflammatory bowel diseases. Cell Res. 2006;16(2):214-19.