RESEARCH ARTICLE

Lack of Prognostic Significance of SOCS-1 Expression in Colorectal Adenocarcinomas

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Abstract

Introduction: Recent studies have indicated that down-regulation of the suppressor of cytokine signaling-1 (SOCS-1) gene results in tumor formation and that SOCS-1 acts as a tumor suppressor gene. SOCS-1 has been also suggested to function as a tumor suppressor with colorectal cancer. Objectives: In the present study, we aimed to determine the association of SOCS-1 expression in colorectal cancer tissues with clinicopathologic characteristics immunohistochemically and also to identify its prognostic significance. Materials and Methods: SOCS-1 expression was studied immunohistochemically in 67 patients diagnosed with resected colorectal carcinomas and 30 control subjects. Results: SOCS-1 expression was found in 46.3% of tumor tissues and 46.7% of the control group. Statistical analyses did not establish any significant association between SOCS-1 expression and clinicopathologic characteristics. Also, no significant association with SOCS-1 expression was found using progression-free survival and overall survival analyses (p=0.326 and p=0.360, respectively). Conclusions: Our results show that SOCS-1 has no prognostic significance in colorectal cancer.

Keywords: Colorectal carcinoma - methylation - suppressor of cytokine signaling-1 - tumour suppressor

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Introduction

Colorectal cancer (CRC) is the third most common cancer in males and second most common in females among all types of cancer. Worldwide, 1.2 million new cases of cancer and 608.700 deaths have been reported in 2008. It still represents a major threat to public health in both developing and developed countries with increasing morbidity and mortality rates (Jemal et al., 2011).

Currently, CRC treatment consists of surgical resection, chemotherapy and radiotherapy. Despite curative therapy, most patients still experience disease relapse within 5 years which lead to morbidity and ultimately mortality (Wilkinson and Scott-Conner 2008; Wolpin and Mayer 2008). Clinical trials for CRC have focused mainly on prevention and early detection of the disease and selection of suitable patients for adjuvant therapy (Walsh and Terdiman 2003; Boursi and Arber 2007; Andre et al., 2009; Lee et al., 2013; Abdel-Rahman et al., 2014).

Survival of CRC patients is directly correlated with the tumor stage at the time of diagnosis. While five-year survival is better for those patients with distant metastases (12%), prognosis is better in those with localized tumor (90%) (Siegel et al., 2011). The most important prognostic indicators in CRC patients include tumor stage and prognostic or predictive markers (Wolpin and Mayer 2008; Colussi et al., 2013; Yang et al., 2014). Candidate markers include blood antigens and circulating tumor cells, tumor enzymes and gene expressions (Denlinger and Cohen 2007; Zlobec and Lugli 2008; Ogino et al., 2009; Chan et al., 2010; Firestein et al., 2010; Imamura et al., 2012; Shima et al., 2011; Fang et al., 2012; Liao et al., 2012; Morikawa et al., 2012; Dong et al., 2013; Li et al., 2013). However, useful prognostic factors related with tumor stage and clinical outcomes of such patients have not been well characterized. Thus, detection of new cancer-related genes which could be used as diagnostic predictors is vital for CRC diagnosis.

Several cytokines are involved in regulation and control of immune responses. Most of these cytokines exert their biological effects via Janus Tyrosine Kinase (JAK) and Signal Transducers and Activators of Transcription (STAT). The Supressors of Cytokine Signalling (SOCS) are a family of intracellular proteins, several of which have emerged as key physiological regulators of cytokine-mediated reactions. The SOCS family consists of eight different members; SOCS-1-SOCS7 and CIS (cytokine-inducible SH2 protein). These proteins have 50-380 aminoacid-long regions with a low level of similarity in their amino terminal residues and all of these eight proteins share two motifs including the 95 aminoacid-long SH2 (src homology domain) domain

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and SOCS box (carboxyl- terminal domain). Additionally, SOCS-1 and SOCS-3 proteins have a kinase inhibitor region known as KIR (Yoshikawa et al., 2001; Oshimo et al., 2004; Watanabe et al., 2004; Melzner et al., 2005; Rakesh and Agrawal., 2005).

Following the discovery of SOCS proteins, negative regulation of cytokine-JAK-STAT pathway has been identified and a number of studies demonstrated the role of SOCS proteins in several immunologic and pathologic conditions (Yoshimura et al., 2005). The suppressor of cytokine signaling-1 (SOCS-1) plays an important role in the physiological regulation of cytokine responses and silencing of SOCS-1 gene by DNA methylation has been established in many human cancers.

In the present study, we aimed to determine the association of SOCS-1 expression and tumor stage with clinicopathologic characteristics and also to identify the prognostic significance of SOCS-1 in surgically resected CRC patients.

Materials and Methods

Study protocol

This study had a retrospective design. Medical files of patients who were followed and treated in our university hospital from 1999 to 2008 were reviewed and pathologic specimens of those patients with adequate data were examined. A total of 67 patients (37 males, 30 females) with primary tumor resection who had been classified using TNM staging system were enrolled in the study.

Thirty subjects (21 males, 9 females) who had undergone intestinal resection due to various reasons (eg., trauma, megacolon) without any underlying malignant or inflammatory conditions were enrolled as control group. Local ethics committee approval was obtained for the conduct of the study.

Immunohistochemical staining

The presence of SOCS-1 expression in tissues was investigated using immunohistochemical staining. Polyclonal anti-rabbit SOCS-1 antibody (ab83493) (Abcam Inc., Cambridge, UK) was used as primary antibody at a 1:100 dilution. Streptovidin-avidin-biotin methodology was used for immunohistochemical staining. Tissue sections 4-microns thick were transferred onto lysine-coated slides and deparaffinized with overnight incubation at 60ºC. They were kept in xylene for 5 minutes in triplicate and 96% citrate buffer (pH 6.0) for 5 minutes in triplicate. Then, they were boiled in microwave oven for 20 minutes at a temperature equal to 750 watts by adding distilled water every 5 minutes. After keeping them at room temperature for 20 minutes, the sections were transferred into PBS (phosphate buffer saline) and rinsed twice with PBS. The sections were dried and kept at humidified room temperature (25ºC) for 15 minutes with application of 3% hydrogen peroxide. Following rinse with PBS once, the sections were kept at protein blocking for 10 minutes and incubated with the primary antibody for 1 hour. Then, they were rinsed twice with PBS for 3 minutes and following application of Biotinylated Link (secondary antibody) for 15 minutes, rinsed with PBS and kept in 3,3' Diaminobenzidine (DAB) chromogen solution for 10 minutes. After rinsing with distilled water, the sections were examined by two experienced pathologists under a light microscope with respect to the extent and intensity of staining. Preparations with substantial staining of the tissue were considered positive (Figure 1, 2, 3, and 4).

Statistical analysis

Mortality data were gathered by making phone calls with the family members of the patients and reviewing the most recent follow-up information. Information on disease recurrence was obtained from outpatient clinics. Overall survival (OS) was defined as the time from surgical resection to death or the last follow-up visit. Progression-free survival (PFS) was considered as the time from surgical operation until the first recurrence after surgery or the last follow-up visit.

Patient data were recorded into the forms previously prepared for the study. Descriptive values for continuous and categorical variables were estimated using mean ±

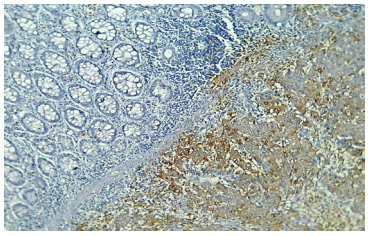

Figure 1. SOCS-1 Negative-Normal Mucosa (DAB X 100)

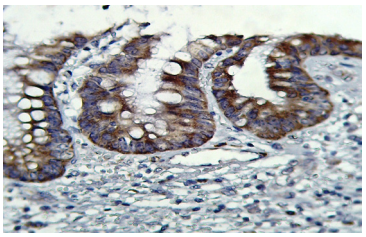

Figure 2. SOCS-1 Positive-Normal Mucosa (DAB X 400)

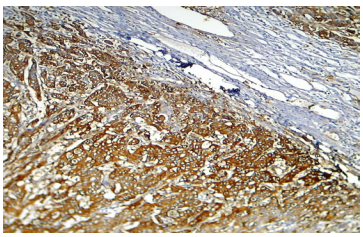

Figure 3. SOCS-1 Strongly Positive-Tumor Tissue (DAB X 100)

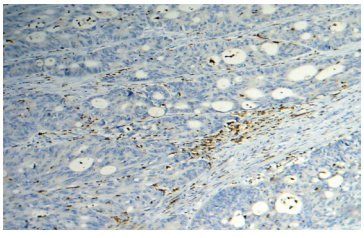

Figure 4. SOCS-1 Negative-Tumor Tissue (DAB X 100)

standard deviation and frequencies (count and percent) respectively. Kolmogorov-Smirnov test was used to determine whether continuous variables showed normal distribution. Independent samples t-test was used to compare patient and control groups for mean age and to compare patients with positive or negative SOCS-1 result with respect to mean total number of resected lymph nodes. The associations of categorical variables with study groups and with SOCS-1 result were examined by an appropriate chi-square analysis. The associations between life expectancy and clinical characteristics were investigated using a Cox regression model and Log-Rank test was used to compare life expectancies of SOCS-1 positive and negative patients. Also, survival curves were plotted for these groups (Figure 5 and 6). During statistical analyses, a result with a p value below 0.05 was considered statistically significant. PASW (SPSS, ver.18) software package was used for statistical analyses.

Results

According to clinicopathologic characteristics of the study group, the mean age was 59.62±12.57 years (youngest patient being 24 years and oldest being 84 years of age) for 67 patients (37 males, 30 females) and 50.9±18.29 years for 30 patients (21 males, 9 females) in the control group (youngest being 25 years and oldest being 83 years of age).

Five patients were stage 1, 22 were stage II, 31 stage were III and 9 were stage IV. Their mean duration of follow-up was 52.9 months. According to WHO classification, 17 (25.4%) had well differentiated adenocarcinoma and 50 (74.6%) had moderately or poorly differentiated adenocarcinoma. Fifty-five (82.1%) cancer patients were T3 stage. Nine patients (13.4%) had metastasis at the time of diagnosis.

Of the colon cancer tissues, 31 (46.3%) were positive for SOCS-1 expression. SOCS-1 expression was positive in 14 subjects (46.7%) in the control group.

Clinicopathologic relationship

As shown in Table 1, no significant association was found between SOCS-1 expression and clinicopathologic characteristics of 67 colorectal cancer patients. Also,

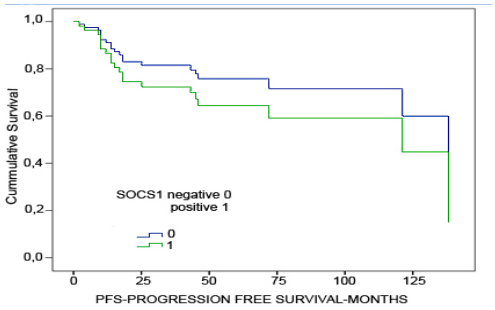

Figure 5. Analysis of Progression Free Survival

Table 1. The Association of Clinicopathologic Characteristics with SOCS-1 among Patients with Colorectal Cancer

Characteristics	Category	SOCS-1 Negative N (%)	SOCS-1 Positive N (%)	p value
Age	≥55	25 (69.4)	24 (77.4)	0.463
	<55	11 (30.6)	7 (22.6)	
Sex	Male	17 (47.2)	20 (64.5)	0.156
	Female	19 (52.8)	11 (35.5)	
Tumor	Rectum	8 (22.2)	8 (25.8)	0.483
Localisation	Sigmoid Colon	18 (50.0)	10 (32.3)	
	Descending Colon	1 (2.8)	2 (6.5)	
	Right Colon	9 (25.0)	11 (35.5)	
Pathological Stage (TNM)	Stage 1+ Stage 2	16 (44.4)	11 (35.5)	0.456
	Stage 3+ Stage 4	20 (55.6)	20 (64.5)	
T Stage	pT1+pT2	4 (11.1)	51 (6.1)	0.548
	pT3+pT4	32 (88.9)	26 (83.9)	
N Stage	N0	17 (47.2)	12 (38.7)	0.483
	N1+N2	19 (52.8)	19 (61.3)	
Metastasis	M0	31 (86.1)	27 (87.1)	0.906
	M1	5 (13.9)	4 (12.9)	
Duke's Classification	A+B	16 (44.4)	11 (35.5)	0.456
	C+D	20 (55.6)	20 (64.5)	
Differentiation	Well	9 (25.0)	8 (25.8)	0.940
	Moderately +Poorly	27 (75.0)	23 (74.2)	
Perineural Invasion	Present	7 (19.4)	6 (19.4)	0.993
	Absent	29 (80.6)	25 (80.6)	
Venous Invasion	Present	4 (11.1)	6 (19.4)	0.345
	Absent	32 (88.9)	25 (80.6)	
Lymphatic Invasion	Present	12 (33.3)	9 (29.0)	0.705
	Absent	24 (66.7)	22 (71.0)	
Tumor Size	<5 cm	18 (50.0)	21 (7.7)	0.142
	≥5 cm	18 (50.0)	10 (32.3)	
Recurrence	Present	10 (27.8)	8 (25.8)	0.856
	Absent	26 (72.2)	23 (74.2)	
Total Lymph Node Removed (Mean±SD)		19.66±12.57	20.16±15.06	0.884

there was no significant difference between patient and control groups in SOCS-1 expression (46.3%, 46.7%, respectively) (p=0.572). In the control group, age, gender and tumor localization did not show statistically significant associations with SOCS-1 expression. Survival analysis showed that age (p=0.002, p=0.003), TNM stage (p=0.009, p=0.009), Duke's stage (p=0.009, p=0.009), pN stage (p=0.015, p=0.014), metastasis (p<0.0001, p<0.0001) and venovascular invasion (p=0.015, p=0.016) determined both overall survival and PFS. There was no significant association of OS and PFS with SOCS-1 expression (p=0.377) (Table 2).

Discussion

Our study is the first to investigate the significance of SOCS-1 expression in human colorectal cancer patients. Prognosis-based treatment planning requires studies investigating whether SOCS-1 predicts prognosis in colorectal cancer. Serial mutations in proto-oncogenes and tumor suppressive genes contribute to the development of a malignant phenotype via different mechanisms. Oncogenic mutations which target signal transmission pathways and signaling proteins are common. Changes in signal transmission abolish the control on the proliferation and/or life functions of the cells. Thus, oncogenic signal transmission has an influential role in the tumor development and invasion/metastatic process. It is thought that since down-regulation of SOCS-1 gene leads to tumor formation, it might act a tumor suppressor gene (Kishimoto and Kikutani 2001; Rottapel et al., 2002). Hypermethylation of the SOCS-1 gene which was first identified by Yoshikawa et al. (2001) in hepatocellular carcinoma was subsequently shown in several solid tissue tumors. Hypermethylation of the SOCS-1 gene was reported in pancreatic ductal neoplasia by Fukushima et al. (2003), in human hepatoblastoma by Nagai et al. (2003), in juvenile colorectal cancers by Fujitake et al. (2004), in colorectal cancer by Lin et al. (2004), in human gastric carcinoma by Oshimo et al. (2004), in breast cancer by Sutherland et al. (2004) and in human gastric cell lines by To et al. (2004).

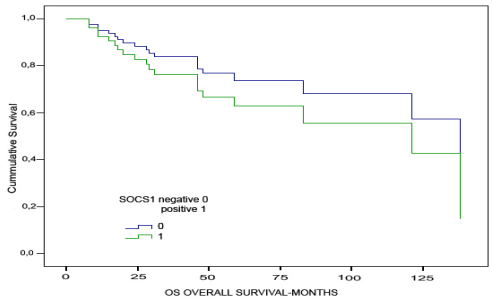

Figure 6. Analysis of Overall Survival

At least two pathways are involved in the sporadic colorectal cancer; one of them is chromosomal instability (CIN) pathway and the other is CpG island methylator phenotype (CIMP) (Issa 2008). CIN pathway accounts for nearly 80% of all colorectal cancers. This pathway confers genetic instability which is required to maintain aneuploidy and adenoma-carcinoma sequence (Grady and Carethers 2008). In colorectal cancer, DNA methylation involves two main changes: hypomethylation and hypermethylation. In cancer, methylated genes have been

Table 2. The Association of Clinicopathologic Characteristics and SOCS-1 Expression with Progression-free Survival and Overall Survival in Patients with Colorectal Cancer

	Progression free survival		Overall survival	
	HR* (95% CI for HR)	P value	HR* (95% CI for HR)	P value
Age	1,080 (1.026 - 1.137)	0.003	1,089 (1.033-1.148)	0.002
Sex (reference = female)	1,685 (0.659- 4.307)	0.276	1,800 (0.702-4.614)	0.221
TNM stage (reference = 1+2)	14,801 (1.96- 111.47)	0.009	14,867 (1.974-111.982)	0.009
T stage (reference = pT1+pT2)	2,093 (0.474 - 9.238)	0.329	2,062 (0.467-9.107)	0.340
N stage (reference = 0)	4,705 (1.364 - 16.24)	0.014	4,636 (1.343-16.003)	0.015
Metastasis (reference = 0)	11,387 (4.22-30.75)	<0.0001	11,610 (4.106-32.832)	<0.0001
Duke's stage (reference = 1+2)	14,801 (1.96- 111.47)	0.009	14,867 (1.974-111.982)	0.009
Differentiation (reference = moderately+poorly)	1,524 (0.578- 4.016)	0.394	.685 (0.260-1.808)	0.445
Lymphatic invasion (reference = 0)	1,063 (0.420-2.691)	0.897	1,012 .400-2.559	0.980
Perineural invasion (reference = 0)	2,348 (0.888-6.210)	0.085	2,235 (0.846-5.908)	0.105
Venovascular invasion (reference = 0)	3,343 (1.249-8.947)	0.016	3,410 (1.269-9.163)	0.015
SOCS-1 expression (reference = 0)	1,580 (0.634-3.938)	0.326	1,535 (0.613-3.843)	0.360

* HR: Hazardratio

characterized as type A (age-related genes) and type C (cancer-specific genes) (Toyota and Issa 1999). In general, type A genes are present in methylated form in both normal and neoplastic tissues. The extent of methylation is proportional to the tissue age (Toyota and Issa 1999; Ahuja and Issa 2000;). Hypermethylation of most type A genes may not directly affect colorectal carcinogenesis but may be an indicator of tissue aging (Toyota and Issa 1999; Ahuja and Issa 2000). However, methylation of type C genes is more specific for neoplastic tissues. In contrast to CIN pathway, CIMP cancers are commonly localized in the proximal colon, generate from serrated polyps rather than adenomatous polyps, are more likely to occur in older women and associated with different survival and treatment results (Ogino et al., 2009).

Several oncologic diseases have been associated with increased JAK/STAT activity which is involved in cell proliferation and malignant growth (Chai et al., 1997). Decreased SOCS expression is usually found in hepatocellular, pancreatic, lung, ovary and breast cancers due to SOCS inactivation by gene mutations and deletions or silencing by DNA hypermethylation (Yoshikawa et al., 2001; He et al., 2003; Nagai et al., 2003; Komazaki et al., 2004; Farabegoli et al., 2005). Methylation of so-called Type C genes is more specific for neoplastic tissues. Five Type C markers (IGF2, CACNA1G, NEU-ROG1, SOCS-1, RUNX3) comprise a CIMP panel and have been associated with colon cancer (Ogino et al., 2009).

In the present study, we evaluated SOCS-1 expression in 67 patients with resected colorectal carcinoma and 30 control subjects immunohistochemically. SOCS-1 expression was present in 46.3% of tumor tissues and 46.7% of control group. We did not find statistically significant associations of SOCS-1 expression with clinicopathologic characteristics including age, gender, tumor localization, TNM stage, Duke's stage, extent of differentiation, perineural invasion, lymphatic invasion, venovascular invasion, tumor width, recurrence and metastasis. Also, disease-free and overall survival analyses did not show any significant association. TNM and Duke's stage, existing metastasis, pN stage and venovascular invasion were found to be independent predictors of prognosis as shown by multivariable analyses (Table 2). In one study, Slattery et al. have shown a significant association of JAK2, SOCS-1, STAT1, STAT4 and TYK2 with rectal cancer survival and emphasized the importance of the JAK/STAT-signaling pathway in colorectal cancer (Slattery et al., 2011). Our results suggest that SOCS-1 has no effect on survival. In vitro studies demonstrated that SOCS-1 is capable of inhibiting different signal transmission pathways activated by various cytokines such as IFN-γ, IL-4, IL-6 and IL-12. Thus, these regulatory features of SOCS-1 are not limited to a single, dedicated cytokine signal transmission pathway (Kawazeo et al., 2001). This suggests that there may be different mechanisms involved in the interactions between SOCS-1 and signal transmission pathways in CRC yet to be elucidated.

Lin et al. have suggested that SOCS-1 inactivation and aggressiveness of colorectal cancer based on their study with 7 colorectal cancer patients with aberrant methylation of SOCS-1 and a high level of lymph node involvement (Sutherland, 2004). However, our study did not find an association with SOCS-1 expression in 40 patients with advanced stage CRC.

In light of the fact that almost 20% of all human cancers result from chronic inflammation, Hanada et al. showed development of colorectal cancer in SOCS-1-deficient mice at the age of 6 months due to spontaneous mutations in nuclear beta-catenin and p53 and stated that SOCS- 1 is a candidate tumor suppressor in inflammation-associated colon cancer and increased activation of STAT 3, NF-κB and STAT 1 is seen in the absence of SOCS-1 (Hanada et al., 2006). A question is borne in mind based on the findings above; ie., has the absence of underlying chronic inflammatory intestinal disease in colorectal cancer patients actually affected SOCS-1 expression in the present study? A study on colorectal cancer patients with underlying inflammatory intestinal disease might reach more robust conclusions.

The small sample size of the present study might have affected the results. Further studies with a larger number of patients and controls might provide a clearer understanding of this issue.

References

Abdel-Rahman WM, Nieminen TT, Shoman S, Eissa S, Peltomaki P (2014). Loss of p15INK^{4}b expression in colorectal cancer is linked to ethnic origin. *Asian Pac J Cancer Prev*, **15**, 2083-7.

Ahuja N, Issa JP (2000). Aging, methylation and cancer. *Histol Histopathol*, **15**, 835-42.

Andre T, Boni C, Navarro M, et al (2009). Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. *J Clin Oncol*, **27**, 3109-16.

Boursi B, Arber N (2007). Current and future clinical strategies in colon cancer prevention and the emerging role of chemoprevention. *Curr Pharm Des*, **13**, 2274-82.

Chai SK, Nichols GL, Rothman P (1997). Constitutive activation of JAKs and STATs in BCR-Abl-expressing cell lines and peripheral blood cells derived from leukemic patients. *J Immunol*, **159**, 4720-28.

Chan AT, Baba Y, Shima K, et al (2010). Cathepsin B expression and survival in colon cancer: Implications for molecular detection of neoplasia. *Cancer Epidemiol Biomark Prev*, **19**, 2777-85.

Colussi D, Brandi G, Bazzoli F, Ricciardiello L (2013). Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. *Int J Mol Sci*, **14**, 16365-85.

Denlinger CS, Cohen SJ (2007). Progress in the development of prognostic and predictive markers for gastrointestinal malignancies. *Curr Treat Options Oncol*, **8**, 339-51.

Dong H, Tang J, Li LH, et al (2013). Serum carbohydrate antigen 19-9 as an indicator of liver metastasis in colorectal carcinoma cases. *Asian Pac J Cancer Prev*, **14**, 909-13.

Fang WJ, Zheng Y, Wu LM, et al (2012). Genome-wide analysis of aberrant DNA methylation for identification of potential biomarkers in colorectal cancer patients. *Asian Pac J Cancer Prev*, **13**, 1917-21.

Farabegoli F, Ceccarelli C, Santini D, Taffurelli M (2005). Suppressor of cytokine signalling 2(SOCS-2) expression in breast carcinoma. *J Clin Pathol*, **58**, 1046-50.

Firestein R, Shima K, Nosho K, et al (2010). CDK8 expression in

470 colorectal cancers in relation to beta-catenin activation, other molecular alterations and patient survival. *Int J Cancer*, **126**, 2863-73.

Fujitake S, Hibi K, Okochi O, et al (2004). Aberrant methylation of SOCS-1 was observed in younger colorectal cancer patients. *J Gastroenterol*, **39**, 120-4.

Fukushima N, Sato N, Sahin F, et al (2003). Aberrant methylation of suppressor of cytokine signalling-1 (SOCS-1) gene in pancreatic ductal neoplasms. *Br J Cancer*, **89**, 338-43.

Grady WM, Carethers JM (2008). Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterol*, **135**, 1079-99.

Hanada T, Kobayashi T, Chinen T, et al (2006). IFN gamma dependent, spontaneous development of colorectal carcinomas in SOCS-1-deficient mice. *J Exp Med*, **203**, 1391-7.

He B, You L, Xu Z, et al (2003). SOCS-3 is frequently silenced by hypermethylation and suppresses cell growth inhuman lung cancer. *Proc Natl Acad Sci USA*, **100**, 14133-8.

Imamura Y, Morikawa T, Liao X, et al (2012). Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers. *Clin Cancer Res*, **18**, 4753-63.

Issa JP (2008). Colon cancer: it's CIN or CIMP. *Clin Cancer Res*, **14**, 5939-40.

Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Kawazoe Y, Naka T, Fujimoto M, et al (2001). Signal transducer and activator of transcription (STAT)-induced STAT inhibitor 1 (SSI-1)/suppressor of cytokine signaling 1 (SOCS-1) inhibits insulin signal transduction pathway through modulating insulin receptor substrate 1 (IRS-1) phosphorylation. *J Exp Med*, **193**, 263-9.

Kishimoto T, Kikutani H (2001). Knocking the SOCS off a tumor suppressor. *Nature Genetics*, **28**, 4-5 .

Komazaki T, Nagai H, Emi M, et al (2004). Hypermethylation-associated inactivation of the SOCS-1gene, a JAK/STAT inhibitor, in human pancreatic cancers. *Jpn J Clin Oncol*, **34**, 191-4.

Lee DW, Han SW, Lee HJ, et al (2013). Prognostic implication of mucinous histology in colorectal cancer patients treated with adjuvant FOLFOX chemotherapy. *Br J Cancer*, **108**, 1978-84.

Li HY, Zhang Y, Cai JH, Bian HL (2013). MicroRNA-451 inhibits growth of human colorectal carcinoma cells via downregulation of Pi3k/Akt pathway. *Asian Pac J Cancer Prev*, **14**, 3631-4.

Liao X, Lochhead P, Nishihara R, et al (2012). Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med*, **367**, 1596-06.

Lin SY, Yeh KT, Chen WT, et al (2004). Promoter CpG methylation of tumor suppressor genes in colorectal cancer and its relationship to clinical features. *Oncol Rep*, **11**, 341-8.

Melzner I, Bucur AJ, Brüderlein S, et al (2005). Biallelic mutation of SOCS-1 impairs JAK2 degradation and sustains phospho-JAK2 action in the MedB-1 mediastinal lymphoma line. *Blood*, **105**, 2535-42.

Morikawa T, Kuchiba A, Liao X, et al (2012). Tumor TP53 expression status, body mass index and prognosis in colorectal cancer. *Int J Cancer*, **131**, 1169-78.

Nagai H, Naka T, Terada Y, et al (2003) Hypermethylation associated with inactivation of the SOCS-1 gene, a JAK/STAT inhibitor, in human hepatoblastomas. *J Hum Genet*. **48**, 65-9.

Nagai H, Naka T, Terada Y, et al (2003). Hypermethylation associated with inactivation of the SOCS-1gene, a JAK/STAT inhibitor, in human hepatoblastomas. *J Hum Genet*, **48**, 65-9.

Ogino S, Nosho K, Irahara N, et al (2009). A cohort study of cyclin D1 expression and prognosis in 602 colon cancer cases. *Clin Cancer Res*, **15**, 4431-38.

Ogino S, Nosho K, Kirkner GJ, et al (2009). CpG island methylator phenotype, microsatellite instability, BRAF mutation and clinical outcome in colon cancer. *Gut*, **58**, 90-96.

Oshino Y, Kuraoka K, Nakayama H, et al (2004). Epigenetic inactiviation of SOCS-1 by CpG island hypermethylation in human gastric carsinoma. *Int J Cancer*, **112**, 1003-9.

Rakesh K, Agrawal DK (2005). Controlling cytokine signaling by constitutive inhibitors. *Biochem Pharmacol*, **70**, 649-57.

Rottapel R, Ilangumaran S, Neale C, et al (2002). The tumor suppressor activity of SOCS-1. *Oncogene*, **21**, 4351-62.

Shima K, Morikawa T, Yamauchi M, et al (2011). TGFBR2 and BAX mononucleotide tract mutations, microsatellite instability, and prognosis in 1072 colorectal cancers. *PLoS One*, **6**, 25062.

Siegel R, DeSantis C, Virgo K, et al (2012). Cancer treatment and survivorship statistics, 2012. *CA Cancer J Clin*, **62**, 220-41.

Siegel R,Ward E, Brawley O, Jemal A (2011). Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin*, **61**, 212-36.

Slattery ML, Lundgreen A, Kadlubar SA, Bondurant KL, Wolff RK (2011). JAK/STAT/SOCS-signaling pathway and colon and rectal cancer. *Mol Carcinog*, **52**, 155-66.

Sutherland KD, Lindeman GJ, Choong DY, et al (2004). Differential hypermethylation of SOCS genes in ovarian and breast carsinomas. *Oncogene*, **23**, 7726-33.

To KF, Chan MW, Leung WK, et al (2004). Constitutional activation of IL-6-mediated JAK/STAT pathway through hypermethylation of SOCS-1 in human gastric cancer cell line. *Br J Cancer*, **91**, 1335-41.

Toyota M, Issa JP (1999). CpG island methylator phenotypes in aging and cancer. *Semin Cancer Biol*, **9**, 349-57.

Walsh JM, Terdiman JP (2003). Colorectal cancer screening: scientific review. *JAMA*, **289**, 1288-96.

Watanabe D, Ezoe S, Fujimoto M, et al (2004). Suppressor of cytokine signalling-1 gene silencing in acute myeloid leukaemia and human haematopoietic cell lines. *Br J Haematol*, **126**, 726-35.

Wilkinson N, Scott-Conner CE (2008). Surgical therapy for colorectal adenocarcinoma. *Gastroenterol Clin North Am*, **37**, 253-67.

Wolpin BM, Mayer RJ (2008). Systemic treatment of colorectal cancer. *Gastroenterology*, **134**, 1296-310.

Yang YF, Zhang XY, Yang M, et al (2014). Prognostic role of nucleophosmin in colorectal carcinomas. *Asian Pac J Cancer Prev*, **15**, 2021-6.

Yoshikawa H, Matsubara K, Qian GS, et al (2001). SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. *Nat Genet*, **28**, 29-35.

Yoshimura A, Nishinakamura H, Matsumura Y, Hanada T (2005). Negative regulation of cytokine signaling and immune responses by SOCS proteins. *Arthritis Res Ther*, **7**, 100-10.

Zlobec I, Lugli A (2008). Prognostic and predictive factors in colorectal cancer. *J Clin Pathol*, **61**, 561-69.