RESEARCH ARTICLE

International Journal of Science, Technology and Society (IJSTS)
Vol. 5, No. 1 & 2 (2020-21): 19-25

Promoter methylation analysis of Suppressor of Cytokine Signaling-1 (SOCS1) gene in Colorectal cancer patients and its association with Clinicopathological characteristics

Alok Kumar[1,2], Pradyumn Singh[2], Anshuman Pandey[3], Gosipatala Sunil Babu[1]

[1]Department of Biotechnology, School of Life Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow-226025
[2]Department of Pathology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow
[3]Department of Surgical Gastroenterology, Dr. Ram Manohar Lohia Institute of Medical Sciences, Lucknow

Publication Info

Article History:
Received: 12-09-2019
Accepted: 17-09-2019
DOI: 10.18091/ijsts.5123

Keywords:
Colorectal cancer (CRC),
Promoter hypermethylation,
Suppressor of cytokine signaling-1 (SOCS1)

Corresponding Author:
Dr. Sunil Babu Gosipatala
Email:
sunil_gos@yahoo.com

ABSTRACT

Colorectal cancer (CRC) is the third most common cancer worldwide with 1.3 million new cases each year. In India, it is the fourth most common cancer in males and the third most common cancer in females. By the advent of molecular biology techniques, new biomarkers such as methylation of DNA, miRNAs resulted and used for early diagnosis as well as personalized therapies. Tumor suppressor gene(s) promoter methylations are one of such biomarkers in identifying the tumor aggressiveness, metastasis and the survival outcome after the surgery. Suppressor of cytokine signaling-1 (SOCS1) gene is a tumor suppressor gene, reported to be silenced in several types of cancers including CRC. However, the role of SOCS1 promoter methylation as a marker remains understudied, not completely established in CRC. We investigated the promoter methylation status of SOCS1 gene in stage II and III CRC samples, find its prognostic significance and association with clinicopathological characteristics. We analyzed 56 CRC samples for SOCS1 promoter methylation and found, 24 samples (42.9%) methylated whereas 32 samples (57.1%) unmethylated. The pathological investigations revealed that SOCS1 promoter methylations were associated with poor differentiation of tumor tissue ($P<0.017$), and decreased the overall survival rate (22.5 months). Further, we also found that promoter methylations are not associated with other clinicopathological characteristics such as lymph node metastasis, tumor stage, and dietary habits.

INTRODUCTION

Colorectal cancer (CRC) is one of the most commonly diagnosed malignant diseases, and is contributing ~ 8% of cancer-associated deaths globally (Bray *et al,* 2018). It is a major health issue in developed nations, and its incidence has increased in developing countries including India globally (Bray *et al,* 2018). Specifically, CRC related mortality is increasing rapidly in many low and middle-income countries (Arnold *et al,* 2017). Further, it is predicted that the incidence of CRC is continue to increase, especially in developing countries due to changing demographics and aging populations (Tsoi *et al,* 2017). So, there is a need for early screening and detection of biomarkers to facilitate early diagnosis and surveillance in CRC cases. Among the biomarkers reported, epigenetic biomarker like DNA methylations are the most frequently used biomarker in various tumors (Okugawa *et al,* 2015) Tumor suppressor gene(s) promoter methylations is one of the epigenetic marker used in identifying the tumor aggressiveness, metastasis and the prediction of survival outcome after the surgery. Promoter methylations in the tumor suppressor genes such as SFRP1 (Kumar, A. *et al,* 2019), IGFBP3 are investigated for their prognostic efficiency. In continuation, researchers studying the suppressor of cytokine signaling-1 (SOCS1) gene promoter methylations and its allelic variants have been linked to different types cancers (Fujitake S *et al,* 2004, Melzner, I. *et al.,* 2005 & 2006). However, in colorectal cancer its methylation status as a biomarker is not well established. The SOCS1 belong to a family of adaptor proteins that negatively regulate cellular signaling. It is known as an inhibitor of Janus

family of tyrosine kinases (JAK), signal transducers and activators of transcription (STAT) pathway (Kile, B.T., *et al* 2001, Davey, G.M., *et al,* 2006 Shi, J. *et al*, 2012). JAK/STAT pathway plays a pivotal role in many cancers including colorectal cancer (Pancik, J., *et al,* 2016, Thomas S.J., *et al.,* 2015). SOCS1 mediate its tumor suppressor functions by diverse mechanisms such as inhibiting the JAK-STAT signalling pathway, promoting the tumor suppressor functions of p53, attenuating MET receptor tyrosine kinase signalling and blocking the oncogenic potential of the cell cycle inhibitor p21. Aberrant methylations of the promoter region of SOCS1 gene and allelic mutations have been linked to different types of malignant cancers (Fujitake S *et al,* 2004, Melzner, I. *et al ,* 2005 & 2006). Epigenetic inactivation due to CpG island methylation of SOCS1 is linked to various cancers such as hepatocellular carcinoma, human gastric carcinoma, melanoma, pancreatic ductal neoplasm and acute myeloid leukemia (Franke S. *et al,* 2001, Yoshikawa, H. *et al.* 2001, Chen, C.Y., *et al* 2003, Fukushima N., *et al*, 2003, Galm, O., *et al* 2003, Oshimo, Y. *et al*, 2004 Mottok, A. *et al*, 2007, Liu, S. *et al*, 2008). However, the clinical significance of SOCS1 inactivation in colorectal cancer remains understudied. Here in this study we examined the correlation between SOCS1 gene promoter methylation and tumor differentiation, overall survival, with the goal of testing its utility as a prognostic biomarker in CRC.

MATERIALS AND METHODS

Patients and tissue samples

We enrolled a total of 56 histopathologically confirmed CRC patients (stage II and III) between October 2013 to March 2017. All these patients were underwent curative surgical resection at Dr. Ram Manohar Lohia Institute of Medical Science Lucknow, Uttar Pradesh, India. Demographical, clinicopathological, tumour-related characteristics of patients recorded and shown in Table 1. A total of 56 primary tumor tissue and adjacent normal colon tissue were collected immediately after curative surgical resection and processed for FFPE block formation. The study was ethically approved by the Institutional Ethics Committee, Dr. Ram Manohar Lohia Institute of Medical Sciences Lucknow, and written informed consent has been taken from each patient. Histopathological examination (HPE), staging and grading of tumors were done as per the standard procedure.

DNA extraction and Bisulfite conversion:

DNA extraction was done by using QIAmp DNA FFPE Tissue Kit (Qiagen GmbH, Hilden, Germany) form the 56 histopathological confirmed Formalin-Fixed paraffin Embedded (FFPE) tumour tissue and adjacent non-tumour tissue with a xylene wash for the removal of paraffin. DNA concentration of the isolated sample were determined by using the NanoDrop Spectrophotometer. The methylation status of DNA were performed by using bisulfite- treated DNA followed by the Methylation Specific PCR (MS-PCR). Bisulfite conversion of DNA was done by using commercially available kit Epitect Bisulfite kit (Qiagen GmbH, Hilden, Germany) by following the recommended protocol from the manufacturer. 20µl solution of DNA (500ng- 2µg) was taken for each bisulfite conversion and following recommended protocol final bisulfite converted DNA eluted in 20 µl elution buffer and converted DNA further processed for methylation specific PCR (MS-PCR) analysis within 24 hours (Li, Y. *et al*, 2011).

Methylation-specific Polymerase Chain Reaction (MS-PCR)

The MS-PCR were performed by the method described by Herman *et al*. in 1996 [23]. The primer sequences used for MS-PCR of the SOCS1 promoter, for methylation-specific were forward 5'-TCGTTCGTACGTCGATTATC-3' and Reverse 5'-AAAAAAATACCCACGAACTCG-3'. The unmethylation-specific primer sequences were forward 5'-TATTTTGTTTGTATGTTGATTATTG-3' and reverse 5'-AAACTCAACACACAACCACTC-3' (Fukushima, N. *et al,* 2003). The unmethylated reaction was predicted to yield a product of 122 bp and the methylated reaction a product of 132 bp and these primers sequences were procured from the Integrated DNA Technologies [IDT, USA]. MS-PCR reaction mixture was prepared with 10 µl master mix (AmpliTaq Gold PCR master mix), 2.5µl (~250ng) bisulfite converted DNA 1.5 µl each primer (forward and reverse) 6µl deionized water. PCR cycling conditions was 95^0C for 10 min (initial denaturation) then 35 cycles consisting of three steps: 95°C for 10s (denaturation), 15s at $58/59^0$C (annealing temperature), 68^0C for 10s (extension) followed by a final extension for 2 minutes at 72^0C. For the amplification of methylated *SOCS1* promoter region the annealing temperature was 59^0C, while for unmethylated was 58^0C (Herman, J.G., *et al*, 1996).

Statistical analysis

The statistical analysis was carried out by the SPSS software (version 20). To find the statistical association between clinicopathological features and promoter methylation, all cases were divided into two groups methylated and unmethylated based on methylation status and Chi-square test used. Survival of CRC cases was defined by using Kaplan Meier survival curve and Log-rank test. To evaluate the potential of SOCS1 gene methylation as a prognostic factor, variables were evaluated by using univariate cox proportional hazard model. The P-value ≤ 0.05 is considered as significant.

Clinicopathological characteristics of CRC cases selected for the study

Table 1: The association between methylation status of SOCS1 gene promoter and clinicopathological features of CRC patients

	No of cases	*Promoter Methylation of SOCS1 gene*		*P value*
Variables	*56*	*Absent (32)*	*Present (24)*	
Age				
<50 years	28	14 (50%)	14 (50%)	.395
≥50 years	28	16 (57.1%)	12 (42.9%)	
Sex				
Male	34	18 (52.9%)	16 (47.1%)	.430
Female	22	14 (63.6%)	8 (36.4%)	
Lymph Node metastasis				
Present	29	17 (58.6%)	12 (41.4%)	.817
Absent	27	15 (55.6%)	12 (44.4%)	
Histological grade				
Well	31	22 (71%)	9 (29%)	.017
Moderate	15	8 (53.3%)	7(46.7%)	
Poor	10	2 (20%)	8 (80%)	
Tumour stage				
T2	9	5 (55.6%)	4 (44.4%)	.376
T3	25	12 (48%)	13 (52%)	
T4	22	15 (68.2%)	7 (31.8%)	
TNM stage				
II	27	15 (55.6%)	12 (44.4%)	.817
III	29	17 (58.6%)	12 (41.4%)	
Histological type				
Infiltrating adenocarcinoma	47	27 (57.4%)	20 (42.6%)	.916
Mucinous adenocarcinoma	9	5 (55.6%)	4 (44.4%)	
Tumour location				
Right Colon (Ascending & Transverse)	21	17 (81%)	4 (19%)	0.02
Left colon (Descending & Sigmoid)	20	9 (45%)	11 (55%)	
Rectum	15	6 (40%)	9 (60%)	
Sample				
Tumor tissue	56	32 (57.14%)	24 (42.86%)	0.000003
Normal tissue	56	53(94.6%)	3 (5.4%)	
Alcohol Habit				
Present	16	10 (62.5)	6 (37.5)	.608
Absent	40	22 (55%)	18 (45%)	
Diet				
Veg.	29	14 (48.3%)	15 (51.7%)	.165
Veg.+ Non-Veg.	27	18 (66.7%)	9 (33.3%)	

P values by Chi-Squire test P value is ≤ 0.05 considered as significant

RESULTS

Clinicopathological characteristics

The clinicopathological features of CRC cases are summarized in table 1. Patients age range was 18-76 years, and the average age was 48 years at the time of diagnosis. Among all 56, 34 (60.7%) were male, and 22 (39.2%) were female. Stage wise, 27 (48.2%) cases were stage II, 29 (51.7%) cases stage III at the time of diagnosis, 47 (83.92%) cases had infiltrate adenocarcinoma NOS, 9 (16.7%) had mucinous adenocarcinoma; and location of tumor: 20 (35.7%) cases had tumor in left colon, 21 (37.5%) right colon and 15 (26.28%) cases tumor in rectum. 31 (55.31%) cases had well-differentiated tumor, 15 (26.7%) cases had moderately differentiated tumor and 10 (17.8%) poorly differentiated. Depth of tumor invasion was T4 in 22 (39.2%) cases T3 in 25 (44.4)% and T2 in 9 (16.07%). Among the 56 patients, 16 (28.5%) patients had alcohol intake history (14 were occasional and habitual); 51.75% cases were vegetarian and 48.21% were non-vegetarian

Methylation of status of SOCS1 promoter

Methylation status of *SOCS1* gene promoter was determined by MS-PCR results (Figure 1). If DNA band appears in methylated reaction cases considered as methylated, if DNA band appear in both reactions (methylated as well as unmethylated) case also considered as methylated because unmethylated band appear due to presence of some normal cells in tumor, if DNA band appears only in unmethylated reaction and no methylated band cases considered as unmethylated. 24 (42.98%) cases out of 56 cases were methylated, and 32 (57.1) cases were unmethylated *SOCS1* promoter. 12/27 (44.4%) cases of stage II and 12/29 (41.4 %) cases of stage III had methylated promoter. Patients of >50 year age had 40.7% cases methylated promoter and <50 year age group. 44.7% (16/34). Cases of male 16 (47%) had the methylated promoter were and 8 (36%) female patients had methylated *SOCS1* promoter

Association of Clinicopathological features with promoter methylation of SOCS1-

To determine whether promoter methylation status of SOCS1 gene is associated with patient's clinicopathological characters such as age, gender, tumor location, lymph node invasion, tumor stage, and histological grade, we performed Chi-square test, and results are summarized in Table 1. We found, SOCS1 gene promoter methylation is significantly associated with histological grade of tumor (p=0.017) and tumor location (P=0.02). We found 80% cases poor differentiated primary tumor (Figure 2a and b) had methylated SOCS1 promoter while 46 %, 29% cases had methylated promoter in moderately and well-differentiated tumor respectively. Promoter methylation occurs, very frequently in left colon tumor 11/20 (55%) and rectum tumor 9/15 (60%) while only 4/21 (19%) cases of right colon had methylated promoter. The association of promoter methylation with tumor site was significant (P=0.02). Our data show, there was no association between SOCS1 promoter methylation and patient's age, gender, lymph-node metastasis, tumor depth, histopathological type, alcohol, and dietary habits.

Survival analysis with reference to *SOCS1* genes promoter methylation

Based on the methylation status of SOCS1 gene promoter, methylated and unmethylated groups were defined. While follow-up in 56 patients up to 60 months, 27 patients had died due to disease-related event, 13 patients are alive and 16 patients miss or not able to do follow up during the follow-up period. The mean estimated overall survival (OS) of unmethylated group was

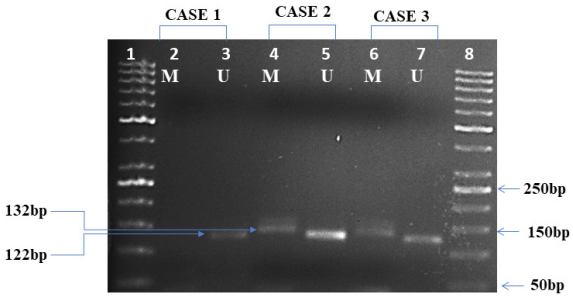

Figure 1: **Methylation-specific PCR results-** Figure showing the result of 3 case CRC of among them two case were methylated (case 2, 3) methylated band appear in lane 4,6 and one unmethylated case (case 1) showing only unmethylated band lane 3. 50bp DNA ladder run in lane 1, 8
M= methylated U= Unmethylated

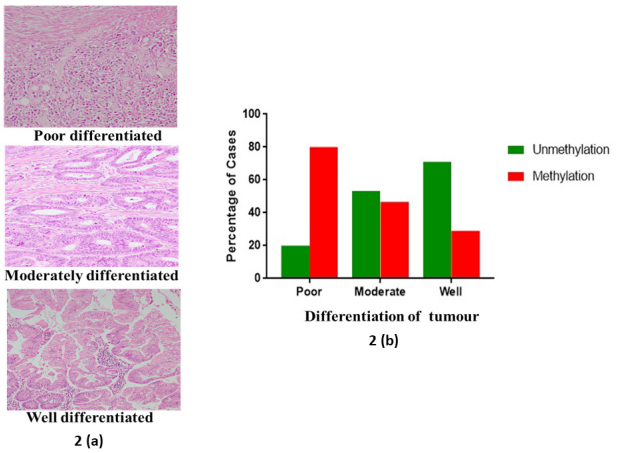

Figure 2(a): Histopathological images represent the poorly, moderately and well differentiation of tumor and **2(b)** Graph showing the percentage of methylated cases and unmethylated case in Poorly, moderately and well-differentiated tumor. Methylation of SOCS1 was closely associated with tumour site (p=0.02 by chi-square test).

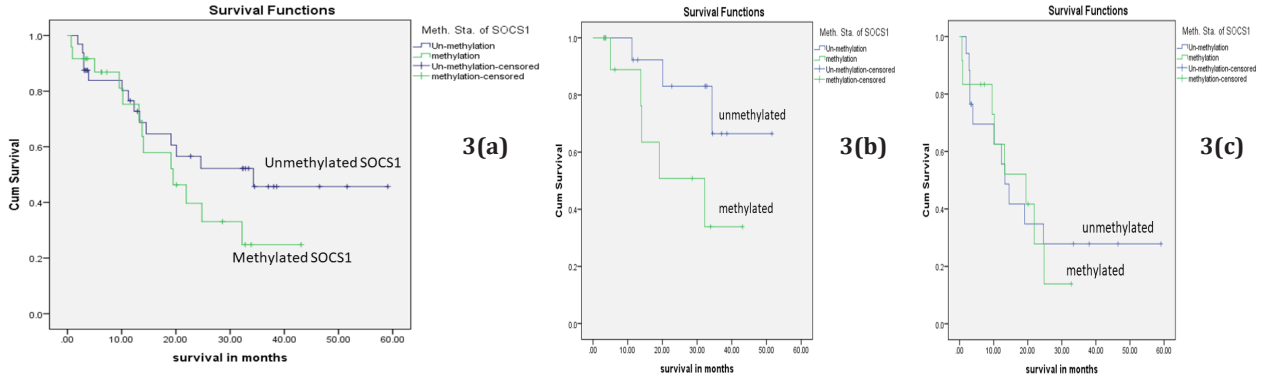

Figure 3: a. Kaplan–Meier survival curve showing the comparison of patients survival with methylated and unmethylated SOCS-1 gene promoter in stage II and III (both) CRC cases. The difference in survival was not significance as P=0.255 (Log Rank test). b- Survival of stage II CRC patients' stage on the basis of methylation status of SOCS1 p=0.067 (Log Rank test) difference in survival was near to significance . c- Survival curve of stage III CRC patients' stage p=0.891 (Log Rank test) .Cum = Cumulative

34.74 months and whereas methylated group OS was 22 months (in stage II and III both cases) (Figure 3a). Unmethylated group survival was better as compared to methylated group (P=0.255 by Log-rank test) and poor survival associated with methylation of SOCS1 promoter. Further, we explored the survival period in the clinical stages, i.e., stage-II and stage-III and found that stage II CRC patients' survival moderately significant differed in methylated and unmethylated group P= 0.067 the survival was 26.5 months, and 42.7 months respectively (survival curve in figure 3b). Though in stage III, survival was 16.4 months, 23.81 months respectively for the methylated unmethylated, group and the difference was not significant (P=0.891) (Figure 3c).

We analyzed stage II CRC cases survival data in multivariate analysis with reference of 7 different clinicopathological features of CRC cases, i.e. age group, gender, tumor stage, tumor differentiation, location of tumor and histological type of tumor. The finding showed that SOCS1 gene is not an independent prognostic marker for stage II CRC cases (HR=2.527, 95% CI: 0.179 – 35.708 p=0.493). Multivariate analysis in stage III CRC cases (HR= 1.082, 95% CI: 0.383 – 3.054 p=0.881) also showed SOCS1 not working as an independent prognostic marker for stage III CRC cases.

DISCUSSION

Aberrant methylations in the promoter region of tumor suppressor genes as a prognostic epigenetic biomarker are being considered in the field of cancer therapy and diagnostics. These epigenetic biomarkers are reported to be associated with tumor differentiation, lymph node metastasis and survival period of the cancer patients before and after curative surgery. Promoter hypermethylation was reported in the tumor suppressor genes such as SFRP1 (Jiang, B.G. *et al*, 2017, Kumar, A. *et al*, 2019) IGFBP3 (Ng, J.M., *et al*, 2015) in colorectal cancer. SOCS1 exhibits tumor-suppressive activity, downregulation of this gene by promoter methylations promotes cancer progression, and this was reported in many studies (Franke S. *et al,* 2001, Yoshikawa, H. *et al.* 2001, Chen, C.Y., *et al* 2003, Fukushima N., *et al*, 2003, Galm, O., *et al* 2003, Oshimo, Y. *et al*, 2004 Mottok, A. *et al*, 2007, Liu, S. *et al*, 2008). Promoter methylations lead to the silencing of the gene SOCS1 was reported in multiple myeloma (Galm, O. *et al*, 2003), AML (Wanatabe, D. *et al*, 2004) and CML (Chim,C.S., *et al*, 2004, Liu, T.C. *et al*, 2003). Promoter methylations of SOCS1 and its association with clinical features were reported by Fujitake, S., *et al*. (2004) in colorectal cancer patients (Fujitake, S. *et al*, 2004). However, the prognostic significance of methylation of SOCS-1 is unknown in Indian CRC patients. So, in this study, we analyzed the promoter methylations of the SOCS1 gene, and its association with the clinicopathological characteristics; possible prognostic relevance among the Indian colorectal cancer patients.

The results indicate that among the 56 colorectal cancer samples, 42.9% (24/56) were methylated and 57% (32/56) were unmethylated. But for normal tissue the methylations observed is only 5.3% (3/56). This indicates that in CRC patients SOCS1 promoter methylations significantly increased. Reports pertaining to the association of promoter methylations and its association with clinicopathological characters and clinical outcome is reported in many cancers including colorectal cancer. So, in our study we didn't find any significant association of clinicopathological characters such as lymph node metastasis, T stage, age, gender, except histological grade and tumor site. However, the survival period is inversely associated with the methylation status of SOCS1 in stage II CRC patients. Our results are in concordance with the results of Chaubey *et al* in where SOCS1 promoter methylations are inversely proportional to overall survival period and tumor progression (Fujitake, S. *et al*, 2004). In our study, we observed the shorter survival period in patients with methylated SOCS1 promoter in stage II CRC and stage III CRC. Similar results were seen in Acute

myeloid leukemia patients where the promoter methylations of SOCS1 has no impact on the overall survival (Tobelaim, W.S. *et al*, 2015). Further studies with large number of sample size need to be conducted for confirmation of clinical use of SOCS1 gene for methylation as a prognostic marker.

ACKNOWLEDGMENTS

Authors would like to thank the funding agencies, University Grant Commission(UGC), New Delhi for UGC-Non-NET fellowship (2013-17) and UGC- National fellowship for SC (RGNF-F1-17.1-2017-18/RGNF-2017-18-SC-UTT-30798/(SA-III/Website) for Mr. Alok Kumar. Authors also like to thank Mr. Manoj Pandey for statistical analysis and Mr. Abhishek Pandeya for helping in the manuscript preparation.

REFERENCES

1. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A and Bray F (2017). Global patterns and trends in colorectal cancer incidence and mortality. *Gut*, *66*(4), 683-691.
2. Bray F, Ferlay J, Soerjomataram I, Siegel R L, Torre LA and Jemal A (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *68*(6), 394-424..
3. Chen CY, Tsay W, Tang JL, Shen HL, Lin S W, Huang SY, Yao M, Chen YC, Shen MC, Wang CH and Tien HF (2003). SOCS1 methylation in patients with newly diagnosed acute myeloid leukemia. *Genes, Chromosomes and Cancer*, *37*(3), 300-305.
4. Chim CS, Wong ASY and Kwong YL (2004). Epigenetic dysregulation of the Jak/STAT pathway by frequent aberrant methylation of SHP1 but not SOCS1 in acute leukaemias. *Annals of Hematology*, *83*(8), 527-532.
5. Davey GM, Heath WR. and Starr R (2006). SOCS1: a potent and multifaceted regulator of cytokines and cell-mediated inflammation. *Tissue antigens*, *67*(1), 1-9.
6. Franke S, Wlodarska I, Maes B, Vandenberghe P, Delabie J, Hagemeijer A and De Wolf-Peeters C (2001). Lymphocyte predominance Hodgkin disease is characterized by recurrent genomic imbalances. *Blood*, *97*(6), 1845-1853.
7. Fujitake S, Hibi K, Okochi O, Kodera Y, Ito K, Akiyama S and Nakao A (2004). Aberrant methylation of SOCS-1 was observed in younger colorectal cancer patients. *Journal of gastroenterology*, *39*(2), 120-124.
8. Fukushima N, Sato N, Sahin F, Su GH, Hruban RH, and Goggins M (2003). Aberrant methylation of suppressor of cytokine signalling-1 (SOCS-1) gene in pancreatic ductal neoplasms. *British journal of cancer*, *89*(2), 338.
9. Galm O, Yoshikawa H, Esteller M, Osieka R and Herman JG (2003). SOCS-1, a negative regulator of cytokine signaling, is frequently silenced by methylation in multiple myeloma. *Blood*, *101*(7), 2784-2788.
10. Herman JG, Graff JR, Myöhänen SBDN, Nelkin BD and Baylin SB (1996). Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proceedings of the national academy of sciences*, *93*(18), 9821-9826.
11. Jiang BG, Wang N, Huang J, Yang Y, Sun LL, Pan ZY and Zhou WP (2017). Tumor SOCS3 methylation status predicts the treatment response to TACE and prognosis in HCC patients. *Oncotarget*, *8*(17), 28621.
12. Kile BT and Alexander WS (2001). The suppressors of cytokine signalling (SOCS). *Cellular and Molecular Life Sciences CMLS*, *58*(11), 1627-1635.
13. Kumar A, Gosipatala SB, Pandey A and Singh P (2019). Prognostic Relevance of SFRP1 Gene Promoter Methylation in Colorectal Carcinoma. *Asian Pacific journal of cancer prevention: APJCP*, *20*(5), 1571-1577.
14. Li Y and Tollefsbol TO (2011). DNA methylation detection: bisulfite genomic sequencing analysis. In *Epigenetics Protocols* (pp. 11-21). Humana Press.
15. Liu S, Ren S, Howell P, Fodstad O and Riker AI (2008). Identification of novel epigenetically modified genes in human melanoma via promoter methylation gene profiling. *Pigment cell & melanoma research*, *21*(5), 545-558.
16. Liu TC, Lin SF, Chang JG, Yang MY, Hung SY and Chang CS (2003). Epigenetic alteration of the SOCS1 gene in chronic myeloid leukaemia. *British journal of haematology*, *123*(4), 654-661.
17. Melzner I, Bucur AJ, Brüderlein S, Dorsch K, Hasel C, Barth TF, Leithauser F and Möller P (2005). Biallelic mutation of SOCS-1 impairs JAK2 degradation and sustains phospho-JAK2 action in the MedB-1 mediastinal lymphoma line. *Blood*, *105*(6), 2535-2542.
18. Melzner I, Weniger MA, Bucur AJ, Brüderlein S, Dorsch K, Hasel C, Leithauser F, Ritz O, Dyer MJ, Barth TF and Möller P (2006). Biallelic deletion within 16p13. 13 including SOCS-1 in Karpas1106P mediastinal B-cell lymphoma line is associated with delayed degradation of JAK2 protein. *International journal of cancer*, *118*(8), 1941-1944.
19. Mottok A, Renné C, Willenbrock K, Hansmann ML and Bräuninger A (2007). Somatic hypermutation of SOCS1 in lymphocyte-predominant Hodgkin lymphoma is accompanied by high JAK2 expression and activation of STAT6. *Blood*, *110*(9), 3387-3390.
20. Ng J and Yu J (2015). Promoter hypermethylation of tumour suppressor genes as potential biomarkers in colorectal cancer. *International journal of molecular sciences*, *16*(2), 2472-2496.
21. Okugawa Y, Grady W M and Goel A (2015). Epigenetic alterations in colorectal cancer: emerging biomarkers. *Gastroenterology*, *149*(5), 1204-1225.
22. Oshimo Y, Kuraoka K, Nakayama H, Kitadai Y, Yoshida K, Chayama K and Yasui W (2004). Epigenetic inactivation of SOCS-1 by CpG island hypermethylation in human gastric carcinoma. *International journal of cancer*, *112*(6), 1003-1009.
23. Pencik J, Pham HTT, Schmoellerl J, Javaheri T, Schlederer M, Culig Z, Merkel O, Moriggl R, Grebien F and Kenner L (2016). JAK-STAT signaling in cancer: From cytokines to non-coding genome. *Cytokine*, *87*, 26-36.
24. Shi J and Wei L (2012) Regulation of JAK/STAT signalling by SOCS in the myocardium. *Cardiovasc Res* ;96:345–347.
25. Thomas SJ, Snowden J A, Zeidler M P and Danson SJ (2015). The role of JAK/STAT signalling in the pathogenesis, prognosis and treatment of solid tumours. *British journal of cancer*, *113*(3), 365.

26. Tobelaim WS, Beaurivage C, Champagne A, Pomerleau V, Simoneau A, Chababi W, Yeganeh M, Thibault P, Klinck R, Carrier JC, Ferbeyre G, Ilangumaran S and Saucier C (2015). Tumour-promoting role of SOCS1 in colorectal cancer cells. *Scientific reports*, *5*, 14301.
27. Tsoi KK, Hirai HW, Chan FC, Griffiths S and Sung JJ (2017). Predicted increases in incidence of colorectal cancer in developed and developing regions, in association with ageing populations. *Clinical Gastroenterology and Hepatology*, *15*(6), 892-900.
28. Watanabe D, Ezoe S, Fujimoto M, Kimura A, Saito Y, Nagai H, Tachibana I, Matsumura I, Tanaka T, Kanegane H, Miyawaki T, Emi M, Kanakura Y, Kawase I, Naka T, Kishimoto T (2004). Suppressor of cytokine signalling-1 gene silencing in acute myeloid leukaemia and human haematopoietic cell lines. *British journal of haematology*, *126*(5), 726-735.
29. Yoshikawa H, Matsubara K, Qian GS, Jackson P, Groopman JD, Manning JE, Harris CC and Herman JG (2001). SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. *Nature genetics*, *28*(1), 29.