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Methylation Profiling SOCS2 in Philadelphia-Negative Myeloproliferative Neoplasm

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

The Janus kinase signal transducer and activator of transcription (JAK / STAT) signalling, which is crucial in Philadelphia-negative myeloproliferative neoplasms' (MPNs), is negatively regulated by molecules such as SOCS, CISH, and SHP1. SOCS2 methylation has been studied in MPN with conflicting results. Here we examine the methylation status of SOCS2 by specific methylation-specific polymerase chain reaction (MSP) in cell lines and 130 diagnostic prepheral blood samples from Ph-ve MPN. Furthermore, we tried to explain the mismatch of the methylation frequency by assigning the investigated MSP primers to the respective genes. Methylation was detected in normal controls using SOCS2-MSP primers in the 3 'translated exon sequence, but not with primers around the transcription start site in the 5' untranslated regions (5'UTR). SOCS2 was completely unmethylated in primary MPN samples and cell lines. In contrast, SOCS2 methylation when using MSP primers located at the 5'UTR is rare in all studies.

In conclusion, SOCS2 methylation is rare in Ph-ve MPN. The appropriate MSP primers are important for an accurate estimate of the methylation frequency. The role of SOCS2 methylation in MPN pathogenesis requires further investigation.

Keywords: Myeloproliferative neoplasms; methylation; SOCS2; MSP primer.

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1. INTRODUCTION

Philadelphia negative (Ph-ve) myeloproliferative neoplasms' (MPNs) is a stem cell disease with proliferation of myeloid compartments that leads to the development of various clinical entities such as polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) [1,2]. Recently, the Janus kinase 2 (JAK2) V617F mutation was detected in most PV patients and approximately half of ET and PMF patients, resulting in constitutive activation of the Janus kinase signal transducer and the activator of transcription (JAK / STAT) [3,4]. However, the pathogenesis in those without the JAK2 V617F mutation remains unknown.

The JAK / STAT signaling pathway is important for the transmission of cytokine signals from the cell surface to the nucleus [5]. Binding of cytokines to their related receptors leads to dimerization of receptor complexes and activation of the Janus family of protein tyrosine kinases [6,7], followed by phosphorylation of the cytoplasmic STATs. During phosphorylation, STATs form homo or heterodimers, migrate to the cell nucleus, and activate gene transcription. This JAK / STAT pathway is down-regulated by members of PIAS, JAK inhibitors such as SOCS family proteins, and protein tyrosine phosphatases (PTPs) such as SHP1 [6-8]. The SOCS family comprises eight members, including SOCS 1-7 and CISH, and is characterized by a central domain of Src homology (SH2) flanked by an N-terminal domain of variable length and a conserved 40 amino acid carboxy-terminal "SOCS box". Domain [6,7]. SOCS proteins suppress JAK / STAT signaling by binding with their SH2 domain to phosphotyrosine residues on activated cytokine or JAK receptors [9]. Including CISH, located at 3p21.2; SOCS1, at 16p13.13; SOCS2 at12q22; and SOCS3 at 17q25.3 are further studied in hematologic malignancies.

SOCS1 and SOCS3 can bind to activated cytokine or JAK receptors through the SH2 domain to inhibit the activation of JAK tyrosine kinase. CISH and SOCS2 inhibit STAT activation by binding to phosphorylated tyrosine residues on activated cytokine receptors and compete with STAT or hinder the receptor's STAT binding sites [9]. Members of SOCS are cytokine-inducible negative regulators of cytokine signaling [9]. SOCS1, SOCS2, SOCS3, and CISH can be induced by many cytokines and therefore serve as negative feedback to contain excessive cytokine signaling [9]. SOCS1 and SOCS3 consist of two exons, the first exon is not translated, while SOCS2 consists of three exons, and the first exon is not translated [10-12]. All of these three genes are embedded in a giant CpG island of more than two kilobases extending from the 5′UTR to the 3-translated exon sequence, as shown on the relevant websites in the Roadmap Epigenomics Project. of the NIH from NCBI [13].

SHP1, also known as HCP, SHPTP1, and PTP1C, is a cytoplasmic PTP. with 68 kd [14]. The human SHP1 gene is located on chromosome 12p13, consists of 17 exons, and comprises ~ 17 KB of DNA. It contains two tandem Src homology domains (SH2), a catalytic domain, and a C-terminal tail of approximately 100 amino acid residues [14]. In contrast to the ubiquitous expression of structurally related SHP2, SHP1 is expressed primarily in hematopoietic cells and is considered a putative tumor suppressor gene in lymphomas and leukemias, as it alters the oncogenic and growth-promoting potential of protein tyrosine kinase [14].

Hypermethylation of CpG islands associated with promoters of tumor suppressor genes and, more recently, microRNA [15-17], leading to gene silencing and thus inactivation of tumor suppressor genes, has been linked to the pathogenesis of hematologic malignancies [18]. Furthermore, aberrant DNA methylation of SOCS1, SOCS2, SOCS3, and SHP1 was investigated in Phve MPN with conflicting results [19-23]. A previous study has shown that while normal cells have unmethylated CpG islands around the transcription start site (TSS) at the 5'UTR, CpG sites within the exon coding sequence for SOCS1 are methylated, resulting in indicates the importance of the specific polymerase chain of methylation. reaction (MSP). Primer selection underlines [24,25]. Furthermore, there are insufficient data on CISH methylation in Phve MPN. Therefore, in this study, we examined the methylation profile of SOCS1, SOCS2, SOCS3, CISH, and SHP1 in MPN using MSP primers in both the 5'UTR and the translated exon sequence of the genes. Finally, we tried to explain the unconformity of the methylation frequency of SOCS1 and SOCS3 in MPN by assigning the investigated MSP primers to the respective genes.

2. MATERIALS AND METHODS

2.1 Patient Samples

DNA was extracted from primary peripheral blood samples at diagnosis of 130 patients with Ph-ve MPNs. Diagnosis of Ph-ve MPN including ET, PV, and PMF was based on WHO criteria. MSP on DNA extracted from primary marrow samples at diagnosis was performed on 130 patients with MPNs [ET, N = 53 (40.8%); PV, N = 66 (50.8%) and PMF, N = 11 (8.4%)]. In brief, there were 69 (53.1%) male and 61 (46.9%) female patients with a median age of 67.5 years (range: 28–89 years).

DNA from three normal bone marrow donors and five normal peripheral blood donors was used as a negative control. In contrast, enzymatically methylated control DNA (CpGenome Universal Methylated DNA, Millipore, Billerica, MA, USA) was considered a positive control. The samples were selected based on the availability of DNA and clinical data. The study followed the rules of the Helsinki Declaration.

2.2 Methylation-specific Polymerase Chain Reaction

According to the manufacturer's instructions, DNA extraction from peripheral blood was performed with QIAamp DNA Blood Mini Kit. DNA bisulfite conversion was performed commercially (Epi®Tect Bisulfite Kit, Qiagen, Dusseldorf, Germany). The methylation status of *SOCS2* gene promoter CpG islands was investigated by MSP as previously reported[24]. Primers used for the methylated MSP (M-MSP) and unmethylated MSP (U-MSP) were listed in Table 1.

Two sets of primers, located 5′ (*SOCS2-5′*) and 3′ (*SOCS2-3′*) to the translation start site of *SOCS2* were used in this study. *SOCS2-5′* and *SOCS-3′* primers were used according to the previous study[29]. All MSP were performed in a thermal cycler (9700, Applied Biosystems, Foster City, CA, USA) under the conditions: 95°C for 10 min., followed by specific cycles of 95°C for 30 sec., specific annealing temperature for 30 sec., 72°C for 30 sec. And a final extension of 10 min. At 72°C. The MSP mixture contained 30 ng of bisulfite-treated DNA, 0.2 mM dNTPs, MgCl2, 10pmol of each primer, 19 PCR buffer, and 1 unit of FastStart Taq DNA polymerase (Roche, Mannheim, Germany) in a final volume of 25 ll. Ten microliters of PCR products were loaded onto 6% polyacrylamide gels, electrophoresed, stained with ethidium bromide, and visualized under ultraviolet light [29].

2.3 Statistical Analysis

Anova 2 and t-test were used to compare categorical variables, and ANOVA test was used for continuous variables. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, IBM, New York, NY, USA) version 20.0. *p*. Valueless than .05 was considered statistically significant.

Table1. Primers were used for the methylated MSP (M-MSP) and unmethylated MSP (U-MSP)

Gene	Forward primer (5’ – 3’)	Reverse primer (5’ – 3’)	Tm/cycles/Mg2+	Reference
SOCS 2-5′				Teofiliet al., 2008
M-MSP	TTT TAG GAT TTG ATT AAG GGG ATC	TAC GAA AAA TAA ACG TAC AAA AAC G	55°C /37/2mM 55°C /40/2mM	
U-MSP	TTT TTT AGG ATT TGA TTA AGG GGA TT	CAA AAA ATA AAC ATA CAA AAA CAA		
SOCS 3′				He et al., 2003
M-MSP	TAT ATA TTC GCG AGC GCG GTT T	CGC TAC GCC CAA ATA TTA ACG	56°C /40/2mM 62°C /35/1.5mM	
U-MSP	TGT GGT GGT TGT TTA TAT ATT TGT GAG TGT GGT T	CAA CCA ACA ATA ACC CAC ACT ACA CCC A		

3. RESULTS

3.1 Controls

As mentioned in the section for materials and methods, two sets of MSP primers, one in the 5′UTR and the other in the 3′translated exonsequence, were available SOCS2 (SOCS2-5′ and SOCS2-3′). Using down stream MSP primers inside the translated exonic sequence, i.e., SOCS2-3′, methylation was detected in four of five normal control DNA samples. On the other hand, SOCS2 was unmethylated in all normal control samples but methylated in the positive control DNA. Therefore, we studied methylation of the SOCS2 gene using MSP with primer set in the 5′UTR near the TSS instead of inside the translated exon sequence.

3.2 Samples

SOCS2-5' and ***SOCS-3'*** **methylations** were recognized using Methylated-specific Polymerase chain reaction (MS-PCR). Materials and protocols were mentioned in materials and methods. ***SOCS2-5'*** was detected in 43 cases (33.1%) patients out of 130 patients with myeloproliferative neoplasms (MPNs). among those patients 66 cases (50.8%) were PV, 53 cases (40.8%) were ET and 11 cases (8.4%) were PMF. The prevalence of methylated *SOCS2-5′* were 24 (36.4%) in PV, 16 (30.2%) in ET and 3 (27.3%) in PMF (Table 2). ***SOCS2-3'*** was detected in 39 (23.5%) patients out of 103 patients with myeloproliferative neoplasms (MPNs). Among those patients, 51 cases (49.5%) were PV, 43 cases (41.7%) were ET, and 9 cases (8.8%) were PMF. The prevalence of methylated *SOCS2-3′* were 19 (37.3%) in PV, 18 (41.9%) in ET and 2 (22.2%) in PMF. Summarised methylated and un-methylated *SOCS2-5′* and *SOCS2-3′*are summarised in Table 2.

At the diagnosis, the presence of methylated *SOCS2-5*' was found to be significant with all haematological parameters age (*p*=.005), total WBCs (*p*=.006), total RBCs count (*p*=.014), Hb concentration (*p*=.044), HCT (*p*=.031) and platelets count (*p*=.081) in PV patients. Association of hematological parameters of PV and *SOCS2-5*' methylation summarised in Table 3.

The presence of methylated *SOCS2-5′* was found to be significant with all haematological parameters age (*p*=.015), total WBCs (*p*=.089), total RBCs count (*p*=.051), Hb concentration (*p*=.030), HCT (*p*=.043) and platelets count (*p*=.096) in ET patients. Association of hematological parameters of ET and *SOCS2-5′* methylation summarisedin Table 4.

Table 2. *SOCS2-5'*and *SOCS2-3'* methylationstatus among MPNs

Disease subtypes	***SOCS2-5' methylated***			***SOCS2-3*' methylated**		
	Total No. of subjects	**Un-methylated N (%)**	**Methylated N (%)**	**Total No. of subjects**	**Un-methylated N (%)**	**Methylated N (%)**
PV	66(50.8%)	42	24(36.4%)	51(49.5%)	32	19
ET	53 (40.8%)	37	16(30.2%)	43(41.7%)	25	18
PMF	11(8.5%)	8	3(27.3%)	9(8.7%)	7	2
Total	130(100%)	87	43	103(100%)	64	39

Table 3. Associations of hematologic parameters with the SOCS2-5' methylation in PV patients

PV	***SOCS2-5*'**			
	unmethylated	**methylated**	**Total**	***P*.value**
No of patients	42	24	66	
Age (years, mean±SD)	46.52±6.89	47.33±7.05	46.82±6.91	.005
WBC (×109/L, mean±SD)	23.81±31.93	23.33±31.33	23.64±31.47	.006
RBC (×109/L, mean±SD)	6.62±1.45	6.9167±1.06	6.73±1.32	.014
Hb (g/dL, mean±SD)	15.31±3.10	17.58±3.39	16.12±3.37	.044
Hct (%, mean±SD)	48.95±8.99	53.92±7.37	50.76±8.72	.031
Platelet (×109/L, mean±SD)	406.93±230.31	526.04±736.14	450.24±478.06	.081

Table 4. Associations of hematologic parameters with the *SOCS2-5′* methylation in ET patients

ET	*SOCS2-5′*			
	Unmethylated	**Methylated**	**Total**	***P*.value**
No of patients	37	16	53	
Age (years, mean±SD)	48.78±8.29	46.50±9.47	48.09±8.64	.015
WBC (×109/L, mean±SD)	38.49±60.00	29.00±25.64	35.62±51.98	.089
RBC (×109/L, mean±SD)	4.84±1.07	4.13±1.20	4.62±1.15	.051
Hb (g/dL, mean±SD)	10.87±2.07	9.88±2.99	10.57±2.39	.030
Hct (%, mean±SD)	36.49±6.69	31.88±8.54	35.09±7.519	.043
Platelet (×109/L, mean±SD)	1102.49±348.08	1495.50±782.71	1221.13±542.01	.096

Table 5. Associations of hematologic parameters with the *SOCS2-5′* methylation in PMF patients

PMF	*SOCS2-5′*			
	unmethylated	**methylated**	**Total**	***P*.value**
No of patients	8	3	11	
Age (years, mean±SD)	44.25±9.004	56.00±3.464	47.45±9.448	.074
WBC (×109/L, mean±SD)	48.62±40.14	110.67±21.36	65.55±45.37	.236
RBC (×109/L, mean±SD)	5.38±2.33	4.00±.00	5.00±2.05	.093
Hb (g/dL, mean±SD)	9.38±1.77	11.67±.58	10.00±1.84	.069
Hct (%, mean±SD)	31.00±6.61	38.00±.00	32.91±6.43	.064
Platelet (×109/L, mean±SD)	370.88±367.07	238.67±30.02	334.81±313.55	.136

Table 6. Associations of hematologic parameters with the *SOCS2-3′* methylation in PV patients

PV	*SOCS2-3′*			
	Unmethylated	**Methylated**	**Total**	***P*.value**
No of patients	32	19	51	
Age (years, mean±SD)	45.50±6.94	47.00±5.55	46.06±6.44	.010
WBC (×109/L, mean±SD)	30.44±36.40	17.95±25.67	25.78±33.11	.161
RBC (×109/L, mean±SD)	6.88±1.48	6.47±1.07	6.73±1.34	.019
Hb (g/dL, mean±SD)	16.00±3.84	16.89±3.23	16.33±3.62	.017
Hct (%, mean±SD)	50.50±9.85	51.84±8.52	51.00±9.31	.008
Platelet (×109/L, mean±SD)	515.91±641.49	337.37±158.27	449.39±521.30	.131

Table 7. Associations of hematologic parameters with the SOCS2-3' methylation in ET patients

ET	*SOCS2-3′*			
	Unmethylated	**Methylated**	**Total**	***P*.value**
No of patients	25	18	43	
Age (years, mean±SD)	46.28±7.197	48.67±10.426	47.28±8.661	.016
WBC (×109/L, mean±SD)	48.32±66.811	32.78±36.006	41.81±55.997	.121
RBC (×109/L, mean±SD)	4.5200±1.04563	4.4444±1.29352	4.4884±1.14168	.005
Hb (g/dL, mean±SD)	10.4000±2.34521	10.0000±2.70076	10.2326±2.47691	.012
Hct (%, mean±SD)	34.4800±6.80759	34.0000±8.71780	34.2791±7.56976	.004
Platelet (×109/L, mean±SD)	1119.13±461.	337.37±158.27	449.39±521.30	.131

The presence of methylated *SOCS2-5′* was found to be significant with age (*p*=.074), total RBCs count (*p*=.093), Hb concentration (*p*=.069), and HCT (*p*=.064) in PMF patients. No significant association was detected between total WBCs (*p*=.236) or platelets count (*p*=.136) and methylated *SOCS2-5′* in PMF patients. Association of hematological parameters of PMF and *SOCS2-5′*methylation summarisedin Table 5.

The presence of methylated *SOCS2-3′* was found to be significant with age (*p*=.010), total RBCs counts (*p*=.019), Hb concentration (*p*=.017), and HCT (*p*=.008).no significant association detected between total WBCs (*p*=.161) or platelets count (*p*=.131) and methylated *SOCS2-3′* in PV patients. Association of hematological parameters of PV and *SOCS2-3′*methylationsummarised in Table 6.

The presence of methylated *SOCS2-3′* was found to be significant with age (*p*=.016), total RBCs counts (*p*=.005), Hb concentration (*p*=.012), HCT (*p*=.004), and platelets count (*p*=.047). No significant association detected between total WBCs (*p*=.121) or platelets count (*p*=.131) and methylated *SOCS2-3′* in PV patients. Association of hematological parameters of PV and *SOCS2-3′*methylationsummarised in Table 7.

The presence of methylated *SOCS2-3′* was found to be significant with age (*p*=.054), total WBCs (*p*=.093), total RBCs counts (*p*=.102), Hb concentration (*p*=.012) and HCT (*p*=.013). No significant association was detected between platelets count (*p*=.320) and methylated *SOCS2-3′* in PMF patients. Association of hematological parameters of PMF and *SOCS2-3′*methylationsummarised in Table 8.

Table 8. Associations of hematologic parameters with the *SOCS2-3′* methylation in PMF patients

PMF	*SOCS2-3′*			
	Unmethylated	**Methylated**	**Total**	***P*.value**
No of patients	7	2	9	
Age (years, mean±SD)	48.00±8.12	57.00±7.07	50.00±8.46	.054
WBC (×109/L, mean±SD)	61.14±48.05	45.50±57.28	57.67±46.79	.093
RBC (×109/L, mean±SD)	4.86±2.19	3.50±.71	4.56±2.01	.102
Hb (g/dL, mean±SD)	9.86±2.12	9.50±2.12	9.78±1.97	.012
Hct (%, mean±SD)	32.29±6.73	31.00±9.89	32.00±6.82	.013
Platelet (×109/L, mean±SD)	476.00±311.06	138.50±92.63	401.00±309.49	.320

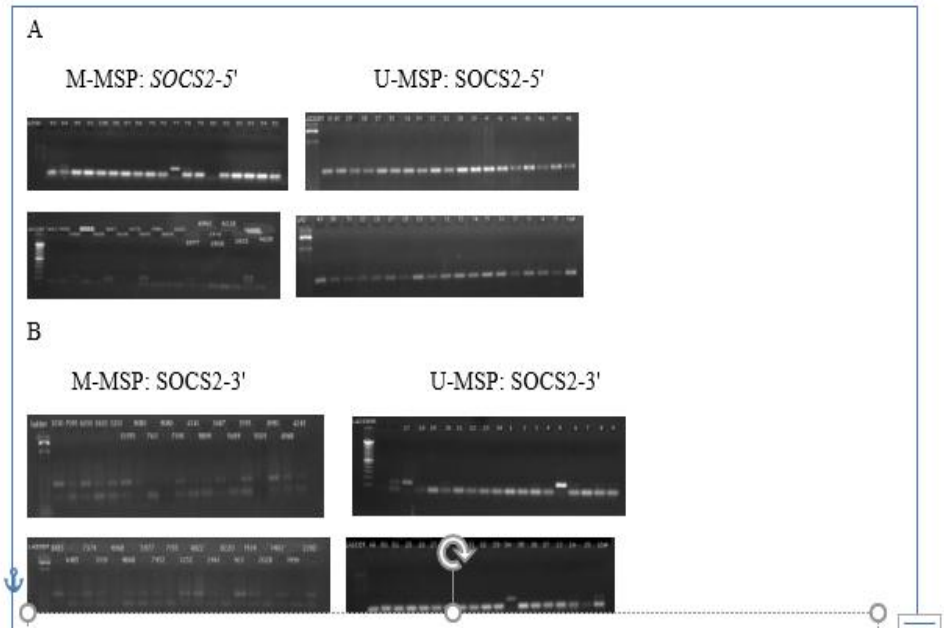

Fig. 1. Methylation-specifie polymerase chain reaction (MSP) of SOCS2-5’ and SOCS2-3’ in MPN primary samples. (A) M-/U-MSP analysis showed that for SOCS2-5’, methylation was present in MPN peripheral blood sample (M: DNA makers; B: blank; PC: Positive control; N: normal control; S: primary sample). (B) M-/U-MSP analysis showed that for SOCS2-3’, methylation was present in MPN peripheral blood samples (M: DNA makers; B: blank; PC: positive control; N: normal control; S: primary sample)

4. DISCUSSION

To explain the discrepancy between the frequency of SOCS1, SOCS2, and SOCS3 methylation in MPN, we mapped the MSP primers used in various studies on the gene map of SOCS1, SOCS2, and SOCS3 and obtained the following observations.

First, the SOCS1, SOCS2, and SOCS3 genes have multiple exons, and the first exon is untranslated. Furthermore, they are all embedded in a huge CpG island with an extension of> 2 kb, extending from the 5'UTR to the exon sequences encoding 3 'translated proteins.

In a previous study, we observed that the use of SOCS13 'MSP primers located in translated exon 2 as described by Watanabe et al. [32], Methylation was detected in six out of 12 normal peripheral blood samples and two out of three normal bone marrow samples [24]. These results were confirmed by sequencing, suggesting that methylation in exon 2 of SOCS1 may not be involved in the regulation of gene transcription. The lack of influence of SOCS1 exon 2 methylation on gene expression was illustrated in HL60 and U937 cell lines, in which the complete methylation of CpG islands within SOCS1 exon 2 was accompanied by significant SOCS1 expression. . connected [32], and thus a lack of correlation between methylation of CpG sites in the 3 'translated exon sequence and gene silencing. In contrast, methylation of CpG islands in the 5'UTR using MSP primers that target CpG sites in the 5'UTR is associated with the inhibition of SOCS1 expression and thus the silencing of SOCS1 by immunohistochemistry. on Linked to hepatoblastoma [33]. Therefore, SOCS1 methylation should be investigated with MSP primers that map to 5'UTR (SOCS15 ') as performed in this study. Using these SOCS15'-MSP primers, no SOCS1 methylation was detected in any of the primary MPN samples or cell lines. Similarly, other studies with primers targeting CpG sites in the 5'UTR showed that none of the total 112 cases of MPN (73 in Fourouclas et al. And 39 in Fernandez-Mercado et al.) Showed SOCS1 methylation. Displayed [21, 22]. In contrast, studies with MSP primers within the 3 'translated exon sequence showed methylation frequencies in the range of 12.8% to 72% (median: 14.5%) [21-23, 29, 34]. Therefore, these results were consistent with the idea that there is a boundary between methylated and unmethylated CpG dinucleotide within a CpG island and that CpG dinucleotide is normally methylated within 3 'translated protein coding sequences in normal cells.

Similarly, SOCS2 methylation has been reported to affect MPN. is frequently methylated [30]. In contrast, we and others found the absence of SOCS2 methylation when we used MSP primers near the TSS at the 5'UTR [19,29]. Quentmeier et al. demonstrated that 28.6% of MPN patients methylated SOCS2 using methylation-sensitive enzymes targeting CpG sites within the 3 'translated exon 2 sequence displayed [30]. In this study, we designed MSP primers mapped to the same exon 2 sequences and confirmed that CpG sites in this region are methylated in normal controls and therefore not suitable for epigenetic regulation of SOCS2 transcription for studies. methylation.

Furthermore, we and others demonstrated rare SOCS3 methylation in MPN using MSP primers near the TSS in the 5'UTR (this study, 0%; Fourouclas et al., 27%; Teofilli et al., 13.5% and Fernandez-Mercado et al., 7.7%) [21, 22, 29], in contrast to the frequent methylation of 41.1% of the samples when MSP primers were used within the translated exon DNA sequence [20]. Our study adopted MSP primers from He et al. [31] close to the TSS for various reasons. First, our MSP primers were located near the TSS in the 5'UTR region. Second, methylation at CpG islands within this region is related to the silencing of SOCS3 and aberrant activation of the JAK / STAT signaling pathway in lung cancer cell lines and is therefore biologically relevant [31]. The use of MSP primers in the 5'UTR was found in patients with PV or ET. no SOCS3 methylation detected [22]. Fourouclas et al. showed SOCS3 methylation in 27% of PMF patients, suggesting disease-specific SOCS3 methylation in MPN [22]. Unfortunately, we had too few PMF patients in our series to verify this finding.

Overall, our results and literature research indicated that MSP primer selection is important in the study of methylation because a methylation boundary can occur in some genes where CpG methylation within the translated exonic DNA sequence is not present. related to gene silencing, it can occur in normal cells. In fact, in a study of the HIC1 gene in normal controls, CpG islet methylation was present in exon 3, which was not associated with HIC1 downregulation and therefore not important for the regulation of HIC1 transcription. In contrast,

CpG sites in the 5'UTR were not methylated in standard bone marrow control, but irreparably methylated in some AML samples, suggesting the importance of methylation of CpGislands prosassociated with 5'UTR rather than the downstream CpG sites in the translated exonic. region in epigenetic regulation of gene expression [35]. Furthermore, the observation that methylation was absent in the 5'UTR of SOCS1 suggested a possible boundary between the methylated and unmethylated sequence in the SOCS1 gene, but was detected in 85% of normal DNA with MSP primers that target to CpG Make in the directed 3′translated exon 2 sequence [21]. Similarly, in SOCS3, methylation using primers near TSS at the 5'UTR was rare, but was present in 41% of primary MPN samples when MSP primers were used within the translated Exon2 sequence [20-22, 29]. Therefore, MSP primers should be chosen in the 5'UTR region, especially near the TSS. Otherwise, methylation unrelated to epigenetic regulation could lead to an erroneously high frequency of gene methylation.

In contrast to the SOCS family, SHP1, another negative JAK / STAT signal regulator, has rarely been tested for methylation in MPN. However, we demonstrated that SHP1 methylation was common in myeloma, resulting in reversible silencing of SHP1 and constitutive activation of JSK / STAT [24]. Here we demonstrate SHP1 methylation in primary MPN samples and K562 cells verified by direct sequencing of MMSP-PCR products. Therefore, SHP1 methylation, leading to reversible gene silencing, is involved in constitutive activation of JAK / STAT in MPN and therefore warrants further studies in larger numbers of patients. Similarly, CISH methylation is absent in MPN.

5. CONCLUSION

In coclusion, it is a comprehensive study of the SOCS2 methylation profile in MPN. Among these, SOCS2 methylation in Phve MPN is rare. A careful selection of MSP primers is also important.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval was obtained from the ethical committee of the Center of Excellence in Genomic Medicine Research (CEGMR) (Bioethical approval code: 01-CEGMR-Bioeth-2019).

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Tefferi A, Essential thrombocythemia, polycythemia vera, and myelofibrosis: current management and the prospect of targeted therapy. American Journal of hematology, 2008;83(6): 491-497.
2. Chim CS, et al. Long-term outcome of 231 patients with essential thrombocythemia: prognostic factors for thrombosis, bleeding, myelofibrosis, and leukemia. Archives of Internal Medicine. 2005;165(22): 2651-2658.
3. Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. Leukemia. 2010;24(6):1128-1138.
4. Vannucchi A et al. Clinical correlates of JAK2 V617F presence or allele burden in myeloproliferative neoplasms: a critical reappraisal. Leukemia, 2008;22(7):1299-1307.
5. Ward AC, Touw I, Yoshimura A, The Jak-Stat pathway in normal and perturbed hematopoiesis. Blood, The Journal of the American Society of Hematology. 2000;95(1):19-29.
6. O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell. 2002;109(2):S121-S131.
7. Alexander WS, Suppressors of cytokine signalling (SOCS) in the immune system. Nature Reviews Immunology. 2002;2(6):410-416.
8. Myers MP, et al. TYK2 and JAK2 are substrates of protein-tyrosine phosphatase 1B. Journal of Biological Chemistry, 2001;276(51):47771-47774.
9. Kubo M, Hanada T, Yoshimura A. Suppressors of cytokine signaling and immunity. Nature Immunology. 2003;4(12):1169-1176.
10. da Cunha Sousa LF, et al. Suppressor of cytokine signaling 2 (SOCS2) contributes to encephalitis in a model of Herpes

infection in mice. Brain Research Bulletin. 2016;127:164-170.
11. Qi S et al. Suppressor of cytokine signaling 1 protects rat pancreatic islets from cytokine-induced apoptosis through Janus kinase/signal transducers and activators of transcription pathway. CHINESE Medical Journal. 2013;126(21): 4048-4053.
12. Persico M et al. Suppressor of cytokine signaling 3 (SOCS3) expression and hepatitis C virus–related chronic hepatitis: insulin resistance and response to antiviral therapy. Hepatology, 2007;46(4): 1009-1015.
13. Bernstein BE et al. The NIH roadmap epigenomics mapping consortium. Nature biotechnology. 2010;28(10):1045-1048.
14. Wu C et al. The function of the protein tyrosine phosphatase SHP-1 in cancer. Gene. 2003;306:1-12.
15. Wang LQ, Liang R, Chim CS. Methylation of tumor suppressor microRNAs: lessons from lymphoid malignancies. Expert review of molecular diagnostics. 2012;12(7): 755-765.
16. Wong KY, Huang X, Chim CS. DNA methylation of microRNA genes in multiple myeloma. Carcinogenesis. 2012;33(9): 1629-1638.
17. Yim, R., et al., DNA Methylation of Tumor Suppressive miRNAs in Non. Frontiers in non-coding RNA. 2012;3(233).
18. Chim C, Liang R, Kwong Y. Hypermethylation of gene promoters in hematological neoplasia. Hematological Oncology. 2002;20(4):167-176.
19. Barrio S, et al. Epigenomic profiling in polycythaemia vera and essential thrombocythaemia shows low levels of aberrant DNA methylation. Journal of Clinical Pathology. 2011;64(11): 1010-1013.
20. Capello D. et al. Epigenetic inactivation of suppressors of cytokine signalling in Philadelphia-negative chronic myeloproliferative disorders. British journal of haematology. 2008;141(4):504-511.
21. Fernández-Mercado, M., et al., Methylation status of SOCS1 and SOCS3 in BCR-ABL negative and JAK2V617F Negative Chronic Myeloproliferative Neoplasms; 2008.
22. Fourouclas N et al. Methylation of the suppressor of cytokine signaling 3 gene (SOCS3) in myeloproliferative disorders. haematologica. 2008;93(11):1635-1644.
23. Abdulkadyrov K, Shuvaev V, Martynkevich I. All we know about polycythemia vera: literature review and own experience. Oncohematology. 2015;10(3):28-42.
24. Chim CS, et al. SOCS1 and SHP1 hypermethylation in multiple myeloma: implications for epigenetic activation of the Jak/STAT pathway. Blood. 2004;103(12): 4630-4635.
25. Chim C, Kwong Y. How frequent is SOCS1 methylation in acute myeloid leukaemia? British journal of haematology. 2004;127(5):609-611.
26. Chim C, Fung T, Liang R. Methylation of cyclin-dependent kinase inhibitors, XAF1, JUNB, CDH13 and soluble Wnt inhibitors in essential thrombocythaemia. Journal of Clinical Pathology. 2010;63(6):518-521.
27. Chim C et al. Methylation of TET2, CBL and CEBPA in Ph-negative myeloproliferative neoplasms. Journal of Clinical Pathology. 2010;63(10):942-946.
28. Chim CS et al. Methylation of miR-34a, miR-34b/c, miR-124-1 and miR-203 in Ph-negative myeloproliferative neoplasms. Journal of Translational Medicine. 2011;9(1):1-10.
29. Teofili L et al. Epigenetic alteration of SOCS family members is a possible pathogenetic mechanism in JAK2 wild type myeloproliferative diseases. International Journal of Cancer. 2008;123(7): 1586-1592.
30. Quentmeier H., et al. SOCS2: inhibitor of JAK2 V617F-mediated signal transduction. Leukemia, 2008;22(12):2169-2175.
31. He B. et al. SOCS-3 is frequently silenced by hypermethylation and suppresses cell growth in human lung cancer. Proceedings of the National Academy of Sciences. 2003;100(24):14133-14138.
32. Watanabe D. et al. Suppressor of cytokine signalling-1 gene silencing in acute myeloid leukaemia and human haematopoietic cell lines. British journal of haematology. 2004;126(5):726-735.
33. Nagai H., et al. Hypermethylation associated with inactivation of the SOCS-1 gene, a JAK/STAT inhibitor, in human hepatoblastomas. Journal of human Genetics. 2003;48(2):65-69.
34. Födermayr M., et al. The clinical impact of DNA methylation frequencies of JAK2 negative regulators in patients with essential thrombocythemia. Leukemia Research. 2012;36(5):588-590.

35. Melki J, Vincent P, Clark S. Cancer-specific region of hypermethylation identified within the HIC1 putative tumour suppressor gene in acute myeloid leukaemia. Leukemia. 1999;13(6): 877-883.

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