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 phosphorytrosine 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 (EpiTect 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. Value less than .05 was considered statistically significant.

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

Table1. Primers were used for the methylated MSP (M-MSP) and unmethylated MSP (U-MSP)
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 exon sequence, 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 SOCS3-3' 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 summarised in Table 4.

| Disease subtypes | Total No. | Un-methylated N (%) | Methylated N (%) | Total No. | Un-methylated N (%) | Methylated N (%) |
|------------------|-----------|---------------------|------------------|-----------|---------------------|------------------|
| PV               | 66 (50.8%)| 42 (36.4%)          | 24 (49.5%)       | 32        | 19                  |
| ET               | 53 (40.8%)| 37 (30.2%)          | 16 (41.7%)       | 25        | 18                  |
| PMF              | 11 (8.5%) | 8 (36.3%)           | 3 (27.3%)        | 7         | 2                   |
| Total            | 130 (100%)| 87 (37.3%)          | 43 (30.2%)       | 64        | 39                  |

Table 2. SOCS2-5' and SOCS2-3' methylation status among MPNs

| PV | SOCS2-5' | | | | | |
|----|----------|----|---|---|---|---|
| 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 3. Associations of hematologic parameters with the SOCS2-5' methylation in PV patients
Table 4. Associations of hematologic parameters with the \textit{SOCS2-5'} methylation in ET patients

| ET      | \textit{SOCS2-5'} | Unmethylated | Methylated | Total | \textit{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 (×10^9/L, mean±SD) | 38.49±60.00       | 29.00±25.64 | 35.62±51.98 | .089  |                 |
| RBC (×10^9/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 (×10^9/L, mean±SD) | 1102.49±348.08    | 1495.50±782.71 | 1221.13±542.01 | .096  |                 |

Table 5. Associations of hematologic parameters with the \textit{SOCS2-5'} methylation in PMF patients

| PMF      | \textit{SOCS2-5'} | unmethylated | methylated | Total | \textit{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 (×10^9/L, mean±SD) | 48.62±40.14       | 110.67±21.36 | 65.55±45.37 | .236  |                 |
| RBC (×10^9/L, mean±SD) | 5.38±2.33         | 4.00±0.00   | 4.59±1.04  | .093  |                 |
| Hb (g/dL, mean±SD) | 9.38±1.77         | 11.67±1.84  | 10.00±1.84 | .069  |                 |
| Hct (%) mean±SD) | 31.00±6.61        | 38.00±0.00  | 34.91±6.43 | .064  |                 |
| Platelet (×10^9/L, mean±SD) | 370.88±367.07     | 238.67±30.02 | 334.81±313.55 | .136  |                 |

Table 6. Associations of hematologic parameters with the \textit{SOCS2-3'} methylation in PV patients

| PV      | \textit{SOCS2-3'} | Unmethylated | Methylated | Total | \textit{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 (×10^9/L, mean±SD) | 30.44±36.40       | 17.95±25.67 | 25.78±33.11 | .161  |                 |
| RBC (×10^9/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  | 16.71±3.62 | .017  |                 |
| Hct (%) mean±SD) | 50.50±9.85        | 51.84±8.52  | 51.00±9.31 | .008  |                 |
| Platelet (×10^9/L, mean±SD) | 515.91±641.49    | 337.37±158.27 | 449.39±521.30 | .131  |                 |

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

| ET      | \textit{SOCS2-3'} | Unmethylated | Methylated | Total | \textit{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 (×10^9/L, mean±SD) | 48.32±66.811      | 32.78±36.006 | 41.81±55.997 | .121  |                 |
| RBC (×10^9/L, mean±SD) | 4.5200±1.04563    | 4.4444±1.29352 | 4.4984±1.14168 | .005  |                 |
| Hb (g/dL, mean±SD) | 10.4000±2.34521   | 10.0000±2.7076 | 10.2326±2.47691 | .012  |                 |
| Hct (%) mean±SD) | 34.16±0.80759     | 34.0000±8.71780 | 34.2791±7.56976 | .004  |                 |
| Platelet (×10^9/L, mean±SD) | 1119.13±461.1     | 337.37±158.27 | 449.39±521.30 | .131  |                 |

The presence of methylated \textit{SOCS2-5'} was found to be significant with age (\textit{p}=.074), total RBCs count (\textit{p}=.093), Hb concentration (\textit{p}=.069), and HCT (\textit{p}=.064) in PMF patients. No significant association was detected between total WBCs (\textit{p}=.236) or platelets count (\textit{p}=.136) and methylated \textit{SOCS2-5'} in PMF patients. Association of hematological parameters of PMF and \textit{SOCS2-5'} methylation summarised in Table 5.
The presence of methylated \textit{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 \textit{SOCS2-3'} in PV patients. Association of hematological parameters of PV and \textit{SOCS2-3'}methylations summarised in Table 6.

The presence of methylated \textit{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 \textit{SOCS2-3'} in PV patients. Association of hematological parameters of PV and \textit{SOCS2-3'}methylations summarised in Table 7.

The presence of methylated \textit{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 \textit{SOCS2-3'} in PMF patients. Association of hematological parameters of PMF and \textit{SOCS2-3'}methylations summarised in Table 8.

### Table 8. Associations of hematologic parameters with the \textit{SOCS2-3'} methylation in PMF patients

| PMF       | 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±7.71  | 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-specific polymerase chain reaction (MSP) of \textit{SOCS2-5'} and \textit{SOCS2-3'} in MPN primary samples. (A) M-/U-MSP analysis showed that for \textit{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 \textit{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 SOCS1 5'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. 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.

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 CpG islands 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 conclusion, 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.

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