Methylation status of *SOCS1* and *SOCS3* in *BCR-ABL* negative and *JAK2*V617F negative chronic myeloproliferative disorders.

To the Editor

BCR-ABL negative Chronic Myeloproliferative Disorders (CMPDs) are a heterogeneous group of clonal haematological malignancies characterised by an abnormal proliferation and survival of one or more myeloid lineage cells that evolve in some cases to an acute myeloid leukemia (AML). These diseases include both classic CMPDs like essential thrombocythemia (ET), polycythaemia vera (PV) and idiopathic myelofibrosis (IMF), and atypical CMPDs (like chronic eosinophilic leukaemia and others). Unlike CML, in which *BCR-ABL* fusion seems to be the primary cause of the disease, the pathogenesis of the other CMPDs have remain elusive until recently. In last years, some genetic alterations have been described, mainly activating some tyrosine kinase genes (like *JAK2* in classic CMPDs, but also *FGFR1*, *PDGFRA* and *PDGFRB* in some atypical CMPDs) but also some of them in genes (like *MPL*) involved in the same signal transduction pathways mediated by the TKs mutated. Of all of these, only V617F mutation in *JAK2* has been found highly recurrent in classic CMPDs and in a minor proportion of atypical CMPDs.

The Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signalling pathway plays a vital role in myeloid differentiation. Cytokines and growth factors bind to the membrane receptors activating a nonreceptor tyrosine kinase, JAK. As noted aboved, one of the four JAKs genes, *JAK2*, has been found mutated in a high proportion of classic CMPDs, and also the gene coding for the thrombopoietin receptor (*MPL*) has been found mutated in some cases with IMF. Once activated JAK, a specific STAT transcription factor is phosphorylated and dimerizes, resulting in transactivation of target genes. Suppressors of cytokine signalling (SOCS) family proteins including SOCS1 and SOCS3 are simultaneously activated and directly interact with JAK in normal cells, resulting in the inhibition of STAT phosphorylation. Thus, SOCS proteins inhibit signal transduction of cytokines and growth factors.

SOCS1 seems to act as a tumor suppressor gene. In fact, methylation and inactivation of this gene has been observed in a variety of human cancers, including around 60% of newly diagnosed AML (1). CML patients also show *SOCS1* methylation that reverts to an unmethylated state during remission (2). *SOCS3* is also frequently methylated and silenced in various cancer types, like human lung cancer (3) and human hepatocelular carcinoma (HCC) (4). Takeuchi *et al* (5) showed that expression of *SOCS3* mRNA in bone marrow cells from CML patients was inversely associated with cytogenetic response to IFN-α.

The aim of this study was to determine the methylation status of CpG islands of *SOCS1* and *SOCS3* genes in CMPD patients under the hypothesis that JAK/STAT pathway could be deregulated in these diseases. The methylation status of 39 CMPD samples was analyzed for *SOCS1* (promoter and two regions in exon 2) and for *SOCS3* (three regions in the promoter). All patients were found to be negative to V617F mutation on JAK2, by cytogenetics and molecular genetics analysis methods at the time of diagnosis. DNA from more than 20 healthy volunteers was used as control samples.

Primers for the promoter region of SOCS1 were reported previously (6); exon 2 primers (regions 1 and 2) of SOCS-1 were designed by us. *SOCS3* primers (three regions) were described by Niwa and colleagues (4): the first set covers two STAT-binding sites that localize upstream to any of the identified transcription start sites [consensus STAT-binding sequence: TT(N)5AA]; the second set spans a DNA region containing three transcription start sites (the second through fourth), and the third set is able to assess DNA methylation in the region containing the third STAT-binding site. PCR products were analyzed on 2% agarose gel.

The promoter region of *SOCS1* was found to be unmethylated in both patient and control samples of our series. Liu *et al* (2) studied *SOCS1* promoter in a CML group and found aberrant hypermethylation in 52% (52/100) of blastic and chronic phase CML samples but no hypermethylation in normal controls or CML in molecular remission. Their results were consistent with the expression analyses. However Hatirnaz *et al* (7) have recently studied *SOCS1* promoter and exon 2 on 56 CML samples and 16 normal samples. They have not detected aberrant methylation in *SOCS1* promoter, the same region analyzed by Liu et al (2). The reasons for this discrepancy proposed

by Hatirnaz *et al* (7) are the different selection of CpG island prediction methods, differences in PCR specificity and efficiency, or a different patient selection criteria and sampling time points.

In contrast, region 1 in *SOCS1* exon 2 was found to be methylated in a high percentage of both patient and control groups (72.2 % and 85.2 % respectively, Table 1). In addition, our region 2 in *SOCS1* exon 2 was unmethylated in patients, and methylated in 8.1% of normal individuals (Table 2). Johan *et al* (8) and Hatirnaz *et al* (7) have also studied the methylation status of exon 2 in AML (40 %, 19/47) and CML (58.9 %, 33/56) respectively, and they have also found methylation in normal controls although in very different proportions (13%, 2/15 and 93.8%, 15/16 respectively). Our results show an important frequency of methylation in healthy controls as observed by Hatirnaz *et al* and no differences were observed with CMPDs samples, supporting the hypothesis that exon 2 is not a pathogenetically relevant region in the study of *SOCS1* methylation status.

Hatirnaz *et al* (7) found *SOCS1* Exon 2 methylated in 15 of 16 healthy controls. We also detected higher methylation in the control group than in patients (Tables 1 and 2). Since the methylation status of Exon 2 is higher in healthy controls than in patients, a hypomethylation status could be speculated in the region studied (7). This hypothesis should be validated across a larger group of healthy individuals, and the results should be confirmed with gene expression and mutation analyses.

Region 1 of *SOCS3* was methylated in 5,3 % (2/38) individuals of the control group and in 7,7 % (3/39) of the CMPDs patients (Table 3). Region 2 of *SOCS3* was found to be completely unmethylated in both patient and control groups. Both results are very different to the dense methylation observed previously in these two regions in non small cell lung cancer (NSCLC) cell lines (3).

However, the methylation status of the third region of *SOCS3* seems to be relevant in CMPDs. We have observed methylation in 23,1 % (9/39) of the CMPDs patients, but in only 5,1 % (2/39) of the control healthy donors [OR= 5,5; (1,114;27,656) 95% CI; *P*=0.023] (Table 4). This result indicates a significant association between methylated status of the region 3 of *SOCS3* gene and the CMPD patient group.

In fact, Niwa *et al* (4) also found methylation in this region 3 of *SOCS3* both in three of the 10 HCC cell lines analyzed and in 33.3% (6/18) HCC primary samples but not in normal samples. In addition, this methylation was associated with subsequent *SOCS3* silencing.

As far as we know, there are no previous reported epigenetic studies of *SOCS1* and *SOCS3* genes of *BCR-ABL* and *JAK2*V617F negative CMPD patients. Our results on methylation status of region 3 of *SOCS3* in these patients are consistent with the results obtained in HCC (4), and support the idea that the analysis of the third region of *SOCS3* is the most relevant for malignant lesions. This hypothesis should be confirmed in other cancer types, and completed with expression studies, in order to firmly establish its significance in the pathogenesis of these diseases.

Acknowledgements

This work has been funded with the help of the Institute of Health Carlos III (FIS PI040037), the PIUNA Program of the University of Navarra and the CajaNavarra Foundation through the Program "You choose, you decide" (Project 10.830)

Table 1. Methylation status of *SOCS1* Exon 2 Region 1

	CMPD patients (n=36)	**Healthy controls (n=27)**	***P***	**OR**	**IC 95%**
***SOCS1* Exon 2 Region 1**					
Unmethylated	10 (27,8%)	4 (14,8%)	0,22	0,45	(0,13;1,64)
Methylated	26 (72,2%)	23 (85,2%)			

Table 2. Methylation status of *SOCS1* Exon 2 Region 2

	CMPD patients (n=39)	**Healthy controls (n=37)**	***P***	**OR**	**IC 95%**
***SOCS1* Exon 2 Region 2**					
Unmethylated	39 (100%)	34 (91,9%)	0,11	Not Computable	Not Computable
Methylated	0 (0%)	3 (8,1%)			

Table 3. Methylation status of *SOCS3* Region 1

	CMPD patients (n=39)	Healthy controls (n=38)	*P*	OR	IC 95%
***SOCS3* Region 1**					
Unmethylated	36 (92,3%)	36 (94,7%)	1,00	1,50	(0,24;9,52)
Methylated	3 (7,7%)	2 (5,3%)			

Table 4. Methylation status of *SOCS3* Region 3

	CMPD patients (n=39)	Healthy controls (n=39)	*P*	OR	IC 95%
***SOCS3* Region 3**					
Unmethylated	30 (76,9%)	37 (94,9%)	0,02	5,55	(1,11;27,66)
Methylated	9 (23,1%)	2 (5,1%)			

References

1. Chen CY, Tsay W, Tang JL, Shen HL, Lin SW, Huang SY *et al*. SOCS1 methylation in patients with newly diagnosed acute myeloid leukemia. *Genes Chromosomes Cancer* 2003; **37**: 300–305.

2. Liu TC, Lin SF, Chang JG, Yang MY, Hung SY, Chang CS. Epigenetic alteration of the SOCS1 gene in chronic myeloid leukaemia. *Br J Haematol* 2003; **123:** 654-661.

3. He B, You L, Uematsu K, Zang K, Xu Z, Lee AY *et al*. SOCS-3 is frequently silenced by hypermethylation and suppresses cell growth in human lung cancer. *Proc Natl Acad Sci* 2003; **100**: 14133–14138.

4. Niwa Y, Kanda H, Shikauchi Y, Saiura A, Matsubara K, Kitagawa T *et al*. Methylation silencing of SOCS-3 promotes cell growth and migration by enhancing JAK/STAT and FAK signalings in human hepatocellular carcinoma. *Oncogene* 2005; **24**: 6406–6417.

5. Takeuchi TK, Sakai I, Narumi H, Yasukawa M, Kojima K, Minamoto Y *et al*. Expression of SOCS3 mRNA in bone marrow cells from CML patients associated with cytogenetic response to IFN-α.. *Leukemia Research* 2005; **29**: 173-178.

6. Nagai H, Naka T, Terada Y, Komazaki T, Yabe A, Jin E *et al.*. Hypermethylation associated with inactivation of the SOCS-1 gene, a JAK/STAT inhibitor, in human hepatoblastomas. *J Hum Genet* 2003; **48:** 65–69.

7. Hatirnaz O, Ure U, Ar C, Akyerli C, Soysal T, Ferhanoglu B *et al*. The SOCS-1 gene methylation in chronic myeloid leukemia patients. *Am J Hematol* 2007; **82**: 729–730.

8. Johan MF, Bowen DT, Frew ME, Goodeve AC, Reilly JT. Aberrant methylation of the negative regulators RASSFIA, SHP-1 and SOCS-1 in myelodysplastic syndromes and acute myeloid leukaemia.. *Br J Haematol* 2005; **129**: 60–5.

Authors

Fernández-Mercado, M.

Cebrián Hernando, V.

Euba Rementeria, B.

García-Granero, M.

Calasanz M.J.

Novo F.J.

Vizmanos Pérez, J.L.

García-Delgado, M.

Department of Genetics, School of Sciences, University of Navarra, Pamplona, Spain

Correspondence:

Prof. Marina García-Delgado, PhD. Department of Genetics, School of Sciences. University of Navarra. C/ Irunlarrea 1, E-31008 Pamplona, Navarra, Spain.

Phone: international +34.948.425600. Fax: international +34.948.425649.

E-mail: mgade@unav.es

Key words

Mieloproliferative Syndromes

Supressor of Cytokine Signaling

SOCS1

SOCS3

Epigenetics

DNA Methylation