Brief report

The *SOCS-1* gene methylation in chronic myeloid leukemia patients

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SOCS-1, an important protein in the JAK/STAT pathway, has a role in the down stream of BCR-ABL protein kinase. We investigated 56 CML patients and 16 controls for the methylation status of *SOCS-1* gene promoter and Exon 2 regions. Exon 2 was found to be methylated in 58.9% of the patients and 93.8% of the controls [*P* = 0.020, OR = 0.121(0.015–0.957)%95CI]. The promoter region was found unmethylated in all patient samples and controls. Although previous studies revealed a relation between *SOCS1* gene Exon-2 hypermethylation and CML development or progression, the results of this study showed no such correlation. On the contrary, our results might be indicating hypomethylation in CML patients, this hypothesis need to be studied in larger study population. Am. J. Hematol. 82:729–730, 2007. © 2007 Wiley-Liss, Inc.

Introduction

SOCS-1 is a member of SOCS (Suppressor of Cytokine Signaling) protein family. Like the other members of this family, suppression activity of SOCS-1 is cytokine specific (leukemia inhibiting factor, interferon γ, growth hormone and prolactin) [1]. This mechanism works as a negative regulator. When SOCS-1 is stimulated by a cytokine, the expression level increases and binds directly to the JAK/STAT complex and inhibit the stimulation [2]. JAK/STATs are also implicated in the downstream of BCR-ABL protein, which activates the JAK/STAT pathway by its constitutive tyrosine kinase activity [3]. It has been shown that the BCR-ABL induces the STAT5 activation, which leads to leukemogenesis [4]. Loss of *SOCS1* gene could play an important role in the regulation and progression of leukomogenesis in CML. Thus, we aimed to study the methylation status of *SOCS1* gene, which has been shown to be methylated in different tumor types [5–7], in CML patients.

Materials and Methods

Patient Samples

Peripheral blood and/or bone marrow samples were collected from 56 CML patients, (33 male, 23 female) and 16 (7 male, 9 female) healthy controls. The mean ages were 46.85 ± 15.41 and 30.37 ± 8.6 for patients and controls, respectively. All patients were found to be t(9;22) positive by cytogenetics and molecular genetics analysis methods at the time of diagnosis. The ethical committee of Cerrahpasa Medical Faculty approved this study and informed consent was obtained from all patients and controls.

Bisulphate Treatment and MS-PCR

Following the DNA isolation NaBiS treatment was performed as described previously [8]. NaBiS treated DNA was purified by the Gene Clean III Kit (Q-Biogene) according to the instructions. After the bisulphate treatment, the samples were amplified both for the promoter and Exon 2 regions by MS-PCR. The primers for the promoter region was reported previously [6] and Exon 2 primers were redesigned by us. The PCR yields were analyzed on 4% agarose gel.

Results and Discussion

Methylation markers are not only shown in solid tumors but are also shown in hematological malignancies like AML and CML.

In this study, 56 CML samples and 16 controls were analyzed for the *SOCS-1* promoter and Exon 2 methylation status. Thirty-three (58.9%) samples were methyated and 23 (41.1%) were unmethylated in CML group (Table I) and 15 (93.8%) were methylated and one was (6.3%) unmethylated in control group [*P* = 0.09, OR 10.455 (1.289–84.785) 95% CI]. In our study we found that the Exon 2 region were methylated in 15 of 16 control samples. Johan et al. also studied the same region and found methylation in healthy controls [9]. Our results are in concordance with Johan et al. and both results support that the Exon 2 is not a relevant region to study *SOCS-1* methylation status and the results can be misleading.

We also studied the promoter region of *SOCS1* gene in CML samples and controls. No methylation was detected in patient or control groups for the promoter region. Liu et al. studied *SOCS1* promoter methylation in CML group [10] and they revealed 52% of methylation. Although, in both studies the same region was analyzed in CML patients with the primers designed from the same CpG island, the outcome was found to be different. The possible reasons for this discrepancy might be due to the following differences; selection of CpG island prediction methods, PCR specificity and efficiency, patient selection criteria and sampling time points.

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TABLE I. Exon2 Methylation Results of CML Patients

Clinical features	Exon2 methylated (*n*)	Exon2 unmethylated (*n*)
Clinical phase		
Cronic	32	20
Blastic	1	3
Ph status		
Positive	33	23
Sex		
Male	20	13
Female	13	10
Sampling time		
Diagnostic sample	6	3
Follow up sample	27	20

According to the results of this study there is no correlation between *SOCS1* methylation and CML development or progression. Since the methylation status of Exon 2 is much higher in healthy controls (15/16) than in patients (33/56) a hypomethylation status could be speculated in the studied region. In the next step, this hypothesis should be studied in a wide range of healthy individuals and the results with gene expression and mutation analyses need to be confirmed.

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