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To the editor:

Silencing of the *SOCS-1* gene by CpG methylation?

There is wide acceptance that methylation of cytosine in cytosine-phosphate-guanosine (CpG)–dense sequences (CpG islands) located within the promoters of many genes plays an important regulatory role in mammalian gene expression, contributing to X-chromosomal inactivation, genomic imprinting, as well as transcriptional regulation of tissue-specific genes during cellular differentiation.[1-3] Besides this epigenetic mechanism of gene silencing by DNA methylation observed in normal cells and tissues, global genomic hypomethylation, accompanied by hypermethylation of CpG islands in promoter regions, is a common characteristic in neoplastic cells.[4] Chromosomal hypomethylation is thought to promote chromosomal instability. Hypermethylation of CpG islands is associated with the inactivation of virtually all pathways involved with the neoplastic process, including DNA repair (eg, *hMLH1, BRCA1, MGMT*), cell cycle regulation (eg, *p16(INK4a), PTEN, RB*), inflammatory/stress response (eg, *COX-2*), and apoptosis (eg, *DAPK, APAF-1*). The methylation status of CpG islands within promoter sequences works as an essential regulatory element by modifying the binding affinity of transcription factors toward its cognate DNA binding sites. Furthermore, transcriptional repression is achieved by proteins (eg, methyl-CpG-binding protein 1, methyl-CpG-binding domain protein 2) that bind to promoter-proximal methylated DNA sequences, thereby maintaining the condensed nucleosomal structure of nontranscribed genes.[5,6]

Recently, *SOCS-1* was described as able to function as a new potential tumor suppressor.[7] The protein families of suppressors of cytokine signaling (SOCS-1 to -7), also known as signal transducer and activator of transcription (STAT)–induced STAT inhibitors (SSIs), are members of cytokine-inducible negative regulators of cytokine signaling. SOCS expression is induced by a subset of cytokines, including interleukin-2 (IL2), IL3, erythropoietin, and interferon-γ.[8] The proteins function downstream of cytokine receptors and take part in a negative feedback loop to attenuate cytokine signaling.

The human *SOCS-1* gene maps to chromosome 16p13.2 and spans about 2.2 kilobase (kb) within a conserved protamine gene cluster. The sequence was submitted to GenBank (accession no. Z46940). A CpG island (441 bp length, 72.8% CG, CG observed/expected 0.86) is located within the promoter region upstream of the untranslated exon 1 of the gene consisting of 2 exons. The SOCS-1 cDNA includes exon 2 coding for a 211–amino acid protein.

We read with interest the article by Galm et al[9] in which these authors show that the expression of the *SOCS-1* gene is silenced by DNA methylation in multiple myeloma. In essence, these data correspond to results published by the same group in *Nature Genetics* suggesting that aberrant methylation of the *SOCS-1* gene correlates with its transcriptional silencing in hepatocellular carcinoma cell lines.[10]

While searching for genes containing CpG-rich promoter sites probably regulated by methylation and involved in cell cycle regulation of B-cell malignancies, we identified *SOCS-1* as a candidate. Controlling the cited primer sequences for methylation-specific polymerase chain reaction of the *SOCS-1* gene by Galm et al,[9] we found them, to our big surprise, located within the coding region of exon 2 and not, as expected, in the CpG island of the *SOCS-1* promoter (Figure 1). As far as we know and as positively stated by the authors,[9] gene silencing by CpG methylation functions via aberrant methylation of promoter sequences with the consequent inhibition of the transcriptional machinery. We kindly ask whether these authors have information inaccessible to us about CpG methylation-sensitive regulatory elements in exon 2 of the *SOCS-1* gene that regulate its expression.

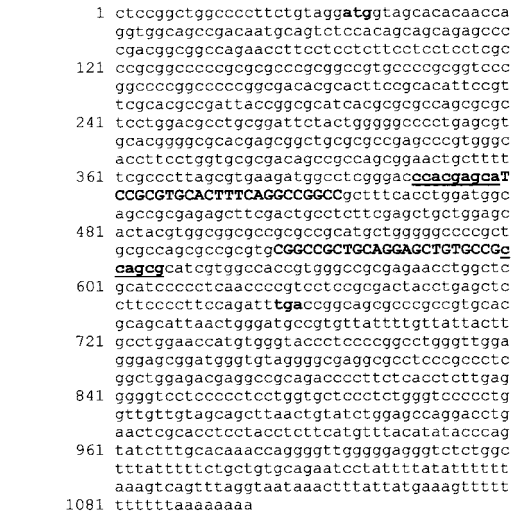

Figure 1. GenBank sequence of the human SOCS-1 mRNA (GenBank accession no. U88326). Start (atg) and stop (tga) of transcription are in bold. The methylation-specific primer sequences cited by Galm et al[9] marked in bold capital letters are located in exon 2. The unmethylation-specific primers are 3′ extended (bold and underlined).

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Response:

CpG island methylation: location matters

Drs Melzner and Möller raise important issues concerning the methylation abnormalities, which are applicable to the epigenetic silencing mechanism in general, described in the *SOCS-1* gene. The authors are correct in finding that the primers that were used for methylation-specific polymerase chain reaction (MSP) are located within what is now termed exon 2 of the *SOCS-1* gene. This location is unusual in that, as their letter indicates, methylation of the promoters of specific genes is associated with gene silencing. Our choice of this region must be understood in the context of how we came to study methylation of the *SOCS-1* gene. Our attention to *SOCS-1* as a potential target of methylation changes was the result of a screening technique called restriction landmark genome scanning (RLGS), which identifies cancer alterations based on 2-dimensional gel analysis. As has been well documented,[1] most cancer alterations are due to methylation of the *Not*I site commonly used in RLGS, as was the case for *SOCS-1*. Since the *Not*I site was located in the region characterized as exon 1 of the *SOCS-1* gene at that time, and our genomic bisulfite sequencing confirmed this methylation change associated with loss of gene expression, this suggested that the location of MSP primers at this *Not*I site would be useful in correlating this epigenetic change with lack of *SOCS-1* expression.[2]

The recently published study[3] extended the analysis of methylation of the cytosine-phosphate-guanosine (CpG) island to multiple myeloma and examined this same region. As demonstrated,[3] methylation of this region of *SOCS-1* was found in multiple myeloma cell lines that did not express *SOCS-1* mRNA and was not present in normal peripheral blood mononuclear cells that express *SOCS-1*. Furthermore, treatment of myeloma cell lines methylated at *SOCS-1* with demethylating agents resulted in restoration of gene expression, demonstrating the importance of methylation changes for gene silencing.[3] However, throughout this report,[3] we were careful to refer to our findings as methylation of *SOCS-1* and as not methylation of the *SOCS-1* promoter.

As the sequencing results of *SOCS-1* methylation demonstrate,[2] it is not a specific methylation pattern that is associated with gene silencing, but a regional increase in methylation that changes chromatin conformation and leads to gene inactivation. In this respect, it is important to recognize that the current "exon 2," which includes the *Not*I site and the MSP primer location, is part of a single, continuous CpG island that extends 5′ to include the newly identified exon 1 and approximately 1000 bp further 5′. Although varying criteria exist for a CpG island, this area of *SOCS-1* with increased CpG frequency incorporates 2.5 bp of sequence. A portion of this region shown in Figure 1 shows the distribution of CpG sites. Thus, methylation of this area of the *SOCS-1* gene studied is not separate from the upstream region where transcription initiates. It remains to be proved as to whether the region studied[3] is the best location to examine methylation changes in the *SOCS-1* gene. However, this region demonstrates tumor-specific methylation[2,3] that correlates with loss of gene expression,[2,3] two important criteria when methylation changes are examined.

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Figure 1. Schematic of the *SOCS-1* CpG island. The black boxes indicate the 2 exons; ATG, the translation start site. Each vertical bar represents a CpG dinucleotide. The arrows show the location of the MSP primers.