



## ORIGINAL ARTICLE

## Methylation Status of SMG1 Gene Promoter in Multiple Myeloma

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## ABSTRACT

**Background:** Epigenetic modifications, such as methylation can occur in multiple myeloma. *SMG1* is an important gene involved in cell growth which defect in methylation of its promoter leads to reduction of cell apoptosis and uncontrolled proliferation. In this study, we identified the methylation status of the *SMG1* gene promoter in patients with multiple myeloma.

**Methods:** Methylation status of *SMG1* promoter in 9 patients with multiple myeloma and 4 healthy subjects as control was determined by Methylation-specific PCR (MSP) method.

**Results:** *SMG1* promoter in all myeloma patients was hemi-methylated. Meanwhile, in healthy subjects, two cases were hemi-methylated and the other two were normal.

**Conclusion:** The results of this study indicated that the prevalence of *SMG1* promoter methylation in patients with multiple myeloma was higher than general population which could be important in understanding the pathogenesis of the disease.

## Introduction

Multiple Myeloma (MM) is the third most common blood cancer after leukemia and lymphoma. It affects approximately 15,000 people every year in the world.<sup>1,2</sup> Epigenetic modifications, defined as biochemical changes of chromatin without permanent alteration in the DNA sequence can be observed at any stage of the tumor. DNA methylation is an example of epigenetic modifications. DNA hypomethylation triggers chromosomal instability and oncogenicity, and hypermethylation leads to the silencing of tumor suppressor genes.<sup>3</sup> *SMG1* is critical in maintaining telomeric integrity, protection against TNF-induced apoptosis and lifespan regulation, and its protein is a member of the PI-3 kinase family.<sup>4,5</sup> Various studies

have shown that *SMG1* gene acts as a new potential tumor suppressor gene in hypoxic tumors and its lowered expression through the CpG islands DNA methylation leads to reduction of apoptosis.<sup>6</sup> Ultimately, it increases the risk of cancers such as hematologic malignancies.<sup>7</sup>

In this study, we aimed to investigate the methylation status of the *SMG1* promoter in MM patients.

## Materials and Methods

In a case-control study; 9 patients with MM referring to Shariati and Shahid Chamran Hospital, and 4 healthy subjects enrolled into this study. The samples were taken after obtaining written informed consent. Peripheral blood smear, along with flow cytometry and cytogenetic

studies were assessed.

#### DNA Extraction

DNA was extracted using GeneAll DNA kit and was analyzed using Nano Drop device. The extracted DNA was treated with high levels of sodium bisulfite using Qiagen EpiTect Bisulfite kit so as to convert unmethylated cytosines to uracil.

#### MSP and Gel Electrophoresis

Methylation-specific PCR (MSP) was performed using two primers. One for methylated DNA (M primer) and one for unmethylated DNA (U Primer) (Table 1). For each sample, two MSP reactions were performed with M primer and U primer. DNA production with M and U primer represents DNA methylation and unmethylation, respectively. The replication of the sample with both M and U primers represents partial methylation (hemi-methylation). Products were separated by electrophoresis on Agarose gel.

## Results

### SMG1 Promoter is Hemi-Methylated in Multiple Myeloma Patients

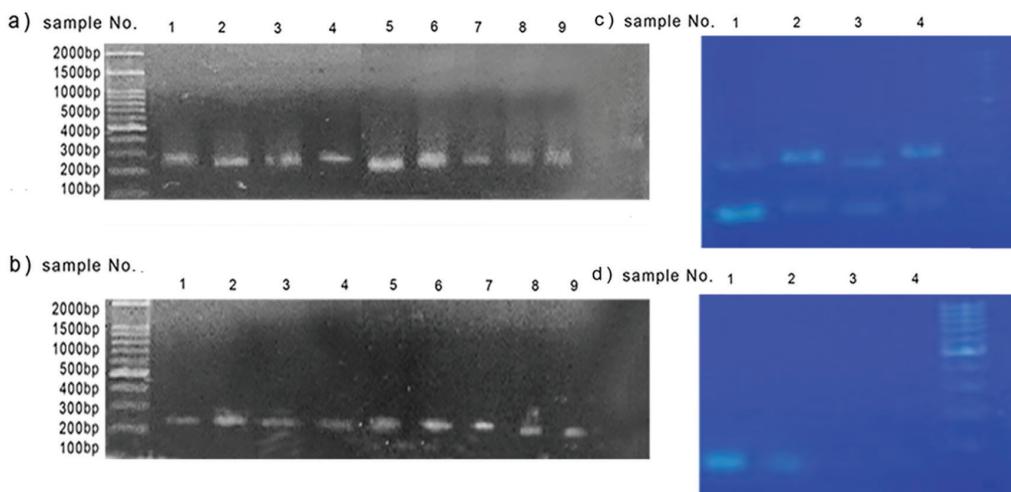
In electrophoresis of the patients' samples (sample 1 to 9), 262 bp DNA bands with both unmethylated (Figure 1-a) and methylated (Figure 1-b) primers were observed at the same time. Thus, the pattern of *SMG1* methylation of patients was hemi-methylated, meaning this genotype is associated with incomplete methylation of promoter regions.

### SMG1 Promoter Status is Hemi-Methylated and Unmethylated in Healthy People

Electrophoresis of healthy samples (samples 1 to 4)

**Table 1:** Sequence of specific methylated and unmethylated primers of SMG1

Gene	Primer types	Primer sequence
SMG1	M-forward	5'-GCGTACGTGAATTAAAGGGTAC-3'
SMG1	M-reverse	5'-AACAAAAAAATCTCCACTACTACGAC-3'
SMG1	U-forward	5'-GGTGTATGTGAATTAAAGGGTATGT-3'
SMG1	U-reverse	5'-AACAAAAAAATCTCCACTACTACAAC-3'



**Figure 1:** Evaluation of SMG1 methylation status in healthy and multiple myeloma patients. a, b) Gel electrophoresis of patient samples with unmethylated and methylated primers, respectively. c, d) Gel electrophoresis of healthy samples with unmethylated and methylated primers, respectively.

showed 262 bp DNA bands with both unmethylated (Figure 1-c) and methylated (Figure 1-d) primers. In samples 1 and 2, both methylated and unmethylated status was present. In these cases, *SMG1* was hemi-methylated. Samples 3 and 4 showed only unmethylated bands.

## Discussion

DNA methylation is one of the best characterized epigenetic modifications. Epigenetic modifications, including DNA methylation, manipulate or affect gene expression. The silencing of tumor suppressor genes is a common phenomenon in the process of malignant transformation. Aberrant methylation of the promoter CpG island of human genes is mostly associated with suppression of gene expression.<sup>8</sup> Our results indicated incomplete methylation of promoter regions of *SMG1* in MM patients. Studies on *SMG1* gene have focused on its suppressive role and extent of its expression in various cancers. In a study on AML patients, it was observed that in 66% of the patients, *SMG1* was hypermethylated.<sup>7</sup> Tiedemann *et al.*, in a research regarding the role of the *SMG1* gene in MM patients showed that *SMG1* is an essential kinase for the survival of MM cells, and its knockdown reduces the survival of myeloma cells.<sup>9</sup> Although the methylation status of *SMG1* has not been studied in MM patients so far, reports of methylation status of this gene has been studied in other cancers. Gubanova *et al.* showed that *SMG1* gene is hypermethylated in head and neck cancers and hence is decreased in expression.<sup>10</sup>

## Conclusion

To date, few studies have been conducted to examine the methylation of *SMG1* gene in hematologic malignancies.

Our results indicated that in patients with multiple myeloma the promoter of the *SMG1* gene is hemi-methylated.

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**Conflict of Interest:** None declared.

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