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DNA methylation analysis of tumor suppressor genes in monoclonal gammopathy of undetermined significance

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Abstract Aberrant DNA methylation is considered an important epigenetic mechanism for gene inactivation. Monoclonal gammopathy of undetermined significance (MGUS) is believed to be a precursor of multiple myeloma (MM). We have analyzed methylation status of $p15^{INK4B}$, $p16^{INK4A}$, ARF, $SOCS-1$, $p27^{KIP1}$, $RASSF1A$, and $TP73$ genes in bone marrow DNA samples from 21 MGUS and 44 MM patients, in order to determine the role of aberrant promoter methylation as one of the steps involved in the progression of MGUS to MM. Methylation specific polymerase chain reaction assay followed by DNA sequencing of the resulting product was performed. $SOCS-1$ gene methylation was significantly more frequent in MM (52%) than in MGUS (14%; $p=0,006$). Methylation frequencies of $TP73$, ARF, $p15^{INK4B}$, $p16^{INK4A}$, and $RASSF1A$ were comparable in MGUS: 33%, 29%, 29%, 5%, and 0%, to that observed in MM: 45%, 29%, 32%, 7%, and 2%. All patients lacked methylation at $p27^{KIP1}$ gene. In both entities, a concurrent methylation of $p15^{INK4B}$ and $TP73$ was observed. The mean methylation index of MGUS was lower (0.16) than that of MM (0.24; $p<0.05$). Correlations with clinicopathologic characteristics showed a higher mean age in MGUS patients with $SOCS-1$ methylated ($p<0.001$); meanwhile in MM, methylation of $p15^{INK4B}$ was more frequent in males ($p=0.009$) and IgG isotype ($p=0.038$). Our findings suggest methylation of $TP73$, ARF, $p15^{INK4B}$, and $p16^{INK4A}$ as early events in the pathogenesis and development of plasma cell disorders; meanwhile, $SOCS-1$ methylation would be an important step in the clonal evolution from MGUS to MM.

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Introduction

Multiple myeloma (MM) is a clonal B-cell neoplasm characterized by atypical plasma cell infiltration in the bone marrow (BM) and the presence of monoclonal protein in serum and/or urine. The natural course of the disease may progress from monoclonal gammopathy of undetermined significance (MGUS) to MM. The transformation rate of MGUS to MM is about 1% per year with an actuarial probability of malignant evolution of 30% at 25 years. After a median of 10 years, about one quarter of MGUS patients develop MM [30]. During this process, multiple genetic alterations are sequentially acquired [23].

There is increasing evidence that, in addition to genetic aberrations, epigenetic processes play a major role in

carcinogenesis, particularly DNA methylation [12]. This modification is imposed only on cytosines that precede guanosines in the CpG dinucleotide. The distribution of the CpG dinucleotides in the genome is asymmetric, showing regions of the highest CpG content, called CpG islands, which are often associated with promoter region. In normal somatic cells, they are usually unmethylated to allow gene expression [2].

In cancer cells, the pattern of methylation is altered. While global hypomethylation of the genome takes place [13], there is a growing list of genes having abnormal methylation at promoter regions [11]. Thus, aberrant methylation of tumor suppressor genes (TSG) promoter regions is now considered an important epigenetic mechanism for gene inactivation [22], in addition to deletion and point mutation. Like genome alterations, hypermethylation occurs in a nonrandom and tumor-type specific pattern [7]. In hematologic neoplasias, repression of gene transcription by aberrant promoter methylation affects genes that regulate different cellular pathways relevant to tumor development, such as cell cycle regulation, DNA repair, apoptosis, and growth factor response, providing a selective advantage to malignant cells, as do mutations.

In recent years, some reports have evaluated methylation status of TSG in different types of solid tumors and hematologic malignancies [3, 24]. However, there is scarce information about aberrant promoter methylation in MGUS. Among them, $p16^{INK4A}$ gene was the most extensively evaluated in this pathology, showing a wide range of methylation frequencies (0–39%) [6, 17, 19, 33, 35, 44, 47]. On the contrary, there are very few information about $p15^{INK4B}$ [19, 33, 44] and ARF [33, 44], only one report for SOCS-1 [41], RASSF1A, and TP73 [44], while $p27^{KIP1}$ has not been evaluated in this entity.

ARF, $p16^{INK4A}$, and $p15^{INK4B}$ genes are cell-cycle regulators located on chromosome 9p21, region that is frequently involved in human tumors. Their alterations results in loss of the normal cell cycle control, promoting malignant transformation [14, 32, 40]. In the same way, $p27^{KIP1}$ gene (12p13) is involved in G1-S transition, inhibiting cyclin-dependent kinase 2 (CDK2)-cyclin E and CDK2-cyclin A [48]. The TP73 gene, banded at 1p36.3, is homologue of the TP53 tumor suppressor. TAp73 is a TP73 isoform with tumor suppressor activity that induces apoptosis and cell-cycle arrest [36, 41]. RASSF1A (3p21.3) may mediate the apoptotic effects of RAS and induce cell cycle arrest by engaging the Rb family cell cycle checkpoint [45, 49]. SOCS-1 (16p13.2) is involved in growth-factor response. This gene is a suppressor of a wide variety of cytokines recently implicated in the negative regulation of IL-6 through direct interaction with JAK (Janus-activated kinase) proteins [27]. In the present study, we have analyzed the methylation status of these seven

tumor-related genes in patients with MGUS and MM in order to determine the role of aberrant promoter methylation as one of the steps involved in the progression of MGUS to MM. Results were correlated with clinicopathologic characteristics of patients.

Material and methods

Patients

Twenty-one patients with MGUS and 44 with untreated MM were included. For all cases, these procedures were performed for diagnostic purposes, and all individuals provided their informed consent according to institutional guidelines. The study was approved by the Ethics Committee of our institution. The diagnosis was based on standard criteria [29]. MM staging was made following the Durie and Salmon criteria [9] and the recently developed International Staging System [18]. Median follow-up was 25.9 months (range 1–101 months). Clinicopathologic characteristics of all patients are summarized in Table 1.

Methylation-specific polymerase chain reaction

Genomic DNA was isolated from BM mononuclear cells. The methylation-specific polymerase chain reaction (MSP) was performed as previously described. Briefly, 1 μg of genomic DNA was denatured at 37°C for 20 min with 3 M NaOH and modified with 4.5 M sodium bisulfite (Sigma, St. Louis) and 10 mM Hydroquinone (Sigma, St Louis) at 55°C for 5 h. After purification with the Wizard™ DNA Clean-up System (Promega A7280, Madison, WI, USA), the DNA was desulfonated in 3 M NaOH. The subsequent PCR amplification was performed using primers designed for either methylated or unmethylated DNA (Table 2). Primers used for ARF, $p15^{INK4B}$, and $p16^{INK4A}$ genes were previously described [10, 21]. Primers for methylated and unmethylated remaining genes were designed taking into account the GeneBank sequences that include the promoter region of the genes TP73 (TAp73 isoform; AC136528), $p27^{KIP1}$ (AF480891) and RASSF1A (NC000003), and exon 2 of SOCS-1 (AF132440). MSPs for SOCS-1, TP73, $p16^{INK4A}$, RASSF1A, and $p27^{KIP1}$ were performed in a thermal cycler TPersonal 48 (Biometra) using Taq Platinum DNA Polymerase (Invitrogen, Brazil) as follows: hot-started at 94°C 2 m and 35 cycles: 94°C 30 s, 30 s at the annealing temperature (Table 2), 72°C 30 s extension, and 72°C 5 m final extension. MSPs for ARF and $p15^{INK4B}$ (using Ampli Taq Gold DNA Polymerase, Perkin Elmer Biosystems and a thermal cycler PTC 150 MJ Research, Watertown, MA, USA) were hot-started at 94°C 10 m, followed by 35 cycles: 94°C 1 m, 1 m at the annealing

Table 1 Clinical characteristics of patients with MGUS and MM

Characteristics	MGUS	MM
No. of cases	21	44
Sex (F/M)	15/6	23/21
Age (years)		
Median (range)	68 (42–86)	67.45 (39–88)
Paraprotein isotype (%)		
IgG	76.2	65.9
IgA	–	26.8
IgM	23.8	2.4
IgM + IgG	–	2.4
Nonsecretory	–	2.4
Type of light chain (%)		
ķ	45.0	66.7
λ	55.0	33.3
ISS		
1	–	25.0
2	–	44.4
3	–	30.6
DS Stage (%)		
I	–	20.0
II	–	14.3
III	–	65.7
BMI (%)		
<30	–	27.3
30–60	–	31.8
>60	–	40.9
$\beta2$ microglobulin (µg/ml; mean (range))	–	2.65 (0.11–12)
LDH (U/l; mean (range))	–	227 (76–536)
Serum albumin (g/dl; mean (range))	–	3.4 (1.7–4.3)
Serum calcium (mg/dl; mean (range))	–	9.3 (8.0–13.2)
Creatinine (mg/dl; mean (range))	–	1.17 (0.60–2.40)
Paraprotein (g; mean (range))	–	3.37 (0.12–9.48)

MGUS monoclonal gammopathy of undetermined significance, *MM* multiple myeloma, *F* female, *M* male, *ISS* International Staging System, *DS* Durie and Salmon, *BMI* bone marrow infiltration, *LDH* lactate dehydrogenase

temperature (Table 2), 72°C 1 m extension, and 72°C 5 m final extension. In the analysis of each gene, we included four types of controls to ensure specificity: peripheral blood samples from ten normal individuals (normal control), CpGenome™ Universal Methylated DNA (Intergen, New York, USA) as positive control; cell lines HCT-116, RAJI, LoVo, and DLD-1; and template (water). Specificity was demonstrated when, for each gene, the M primers only yielded a band from positive control, the U primers produced a band from normal DNA, and none of the primers yielded any product in the absence of template or when nonbisulfite-treated DNA was used as template. Cell lines information about methylation status was obtained from the literature. To confirm the intact nature of the genomic DNA, MSP from patient samples was also performed with the specific primers for the unmethylated condition of each gene.

DNA sequencing

PCR products were purified in 2% agarose gels, sequenced bidirectionally, and analyzed on an automated DNA sequence analyzer (377 ABI Prism, PE Biosystem, Foster City, CA, USA). The specificity of the MSP was verified by DNA sequencing of methylated positive control. DNA sequence of methylated control DNA was aligned and compared with sequence of the wild-type DNA. Methylated cytosine residues in CpG dinucleotide remained as C, whereas unmethylated cytosines were changed to T after bisulfite conversion.

Statistical analysis

Groupwise comparison of the distribution of clinical and laboratory variables was performed with the Student's *t* test

Table 2 Primer sequences, size products, and annealing temperatures

Gene	Sense (5′→3′)	Antisense (5′→3′)	Size product (pb)	Annealing temperature (°C)
ARF U	TTTTTGGTGTTAAAGGGTGGTGTAGT	CACAAAAACCCTCACTCACAACAA	132	63
ARF M	GTGTTAAAGGGCGGCGTAGC	AAAACCCTCACTCGCGACGA	122	60
p15 U	TGTGATGTGTTTGTATTTTGTGGTT	CCATACAATAACCAAACAACCAA	154	63
p15 M	GCGGGTTCGTATTTTGCGGTT	CGTACAATAACCGAACGACCGA	148	63
p16 U	TTATTAGAGGGTGGGGTGGATTGT	CAACCCCAAACCACAACCATAA	151	58
p16 M	TTATTAGAGGGTGGGGCGGATCGC	GACCCCGAACCGCGACCGTAA	150	55
RASSF1A U	GGTTTGTTTTGTGGTTTTAGTTT	CCCCTTCCACTCTCATAAACA	363	55
RASSF1A M	GTTCGTTTTGTGGTTTCGTTC	CCCTTCCGCTCTCGTAAACG	361	55
TP73 U	GAAGGAGGTGGGGTAGAGT	ATCCATCCCAACTAACCTAACA	315	58
TP73 M	AAGGAGGCGGGGTAGAGC	CCGTCCCGACTAACCTAACG	312	58
SOCS-1 U	TGAAGATGGTTTTGGGATTTAT	CTCCAACCAAAAACAAAAAACA	437	58
SOCS-1 M	GAAGATGGTTTCGGGATTTAC	CTCCAACCGAAAACGAAAAACG	435	55
p27 U	GTTTATGGTTTTGTGATTTTGAT	CTCTAAAAACACACAAAAACTCAC	218	55
p27 M	TACGGTTTTGCGATTTCGAC	GTAAAAACGCGCAAAAACTACG	214	55

U unmethylated,: methylated

(for quantitative variables) and the Fisher's exact test (for categorical variables). Overall survival (OS) measured from diagnosis to last follow-up or death and progression-free survival (PFS) measured from the date of the first response to the progression was estimated by the Kaplan–Meier method and compared by the log-rank test. For all tests, $p < 0.05$ was regarded as statistically significant. The analysis was made using SPSS program version 11.5, for Windows. The methylation index (MI; ratio between the number of genes methylated and the number of genes analyzed) was also calculated.

Results

Methylation analysis

We have evaluated methylation status of $p16^{INK4A}$, $p15^{INK4B}$, ARF, $p27^{KIP1}$, TP73, RASSF1A, and SOCS-1 genes in BM cells from patients with MGUS and MM using MSP assay, followed by sequencing analysis of the PCR products. DNA samples from ten healthy blood donors were also analyzed. None of the normal controls showed aberrant methylation of the evaluated genes. The positive and the negative controls showed the expected MSP results (normal DNA: U-MSP positive/M-MSP negative; methylated DNA: U-MSP negative/M-MSP positive).

In MGUS, the MI ranged from 0 to 0.43 with a median of 0.14, corresponding to one gene/sample; 66.7% of patients showed one to three genes aberrantly methylated (Fig. 1a). The analysis of MM patients showed that MI

ranged from 0 to 0.71 with a median of 0.29, corresponding to two genes per sample. The majority of samples (84.1%) showed methylation of at least one gene. Only one gene was affected in 27% cases, and 39% patients presented two methylated genes (Fig. 1b). Whereas, the percentage of MGUS cases with at least one methylated gene was not statistically different to that of MM patients, the mean MI of MM was higher than that of MGUS ($p < 0.05$).

Fourteen percent of MGUS patients showed SOCS-1 methylation, a frequency significantly lower than that observed in MM (52%; $p = 0.006$). As for TP73, ARF, $p15^{INK4B}$, and $p16^{INK4A}$ genes, their frequencies in MGUS were 33%, 29%, 29%, and 5%, respectively, comparable to that observed in MM (45%, 29%, 32%, and 7%, respectively). All MGUS patients showed unmethylated RASSF1A; meanwhile, this gene was methylated in only one MM patient. Both entities showed lack of methylation of $p27^{KIP1}$ gene. A statistical association in the pattern of methylation between $p15^{INK4B}$ and TP73 genes was found in both pathologies ($p = 0.008$ and $p = 0.04$ for MM and MGUS, respectively; Figs. 1a, b). Figure 2a shows MSP assays from the analyzed TSG. Authenticity of MSP was confirmed by sequencing methylated products. Representative sections of the sequencing analysis of different genes are shown in Fig. 2b.

Relation between methylation and clinicopathologic parameters

In both entities, methylation status and clinicopathological features, as described in Table 1, were correlated. In MGUS

Fig. 1 Methylation status in patients with MGUS (**a**) and MM (**b**). *White boxes* represent unmethylated samples and *gray boxes* represent methylated samples. *MI* methylation index

a)   Monoclonal gammopathy of undetermined significance samples

Patient	*ARF*	*p15*^{INK4B}	*p16*^{INK4A}	*RASSF1A*	*SOCS-1*	*TP73*	*p27*	MI
1								0.00
2								0.00
3								0.00
4								0.00
5								0.00
6								0.00
7								0.00
8	■							0.14
9		■						0.14
10						■		0.14
11			■					0.14
12		■						0.14
13	■							0.14
14						■		0.14
15	■				■			0.29
16					■	■		0.29
17		■				■		0.29
18		■				■		0.29
19	■				■			0.29
20	■	■				■		0.43
21	■	■				■		0.43

b)   Multiple myeloma samples

Patient	*ARF*	*p15*^{INK4B}	*p16*^{INK4A}	*RASSF1A*	*SOCS-1*	*TP73*	*p27*	MI
1								0.00
2								0.00
3								0.00
4								0.00
5								0.00
6								0.00
7								0.00
8					■			0.14
9					■			0.14
10					■			0.14
11					■			0.14
12					■			0.14
13						■		0.14
14		■						0.14
15					■			0.14
16					■			0.14
17		■						0.14
18						■		0.14
19	■							0.14
20						■		0.29
21					■			0.29
22					■	■		0.29
23					■	■		0.29
24	■							0.29
25	■							0.29
26		■				■		0.29
27		■				■		0.29
28		■				■		0.29
29		■				■		0.29
30	■					■		0.29
31					■			0.29
32					■	■		0.29
33		■						0.29
34					■			0.29
35								0.29
36	■							0.29
37	■		■		■			0.43
38			■		■	■		0.43
39		■		■		■		0.43
40	■			■		■		0.43
41	■	■				■		0.43
42	■	■			■	■		0.57
43	■	■			■	■		0.57
44	■	■	■		■	■		0.71

Fig. 2 **a** Examples of the MSP assay and sequencing in patients with MGUS (*top*) and MM (*bottom*). MGUS patients: *1* Cases 8 and 13 with *ARF* methylated, sample 1 unmethylated; *2* Patients 9 and 17 with *$p15^{INK4B}$* methylated and patient 2 unmethylated. MM patients: *3* Cases 8, 9, 10, and 11 with *SOCS-1* gene methylated; *4* Patients 13, 18, and 20 with *TP73* gene methylated and sample 24, unmethylated; *5* Patients 37 and 38 with *$p16^{INK4A}$* methylated and patient 1, unmethylated. *M* methylated reaction and *U* unmethylated reaction; *Pos* Positive control (CpGenome Universal Methylated DNA); *Neg* Negative control (water); *MW* molecular weight marker. Products' sizes are showed on the left. **b** Representative sections of the sequencing analysis of *SOCS-1* (*left*) and *TP73* (*right*) genes. The sequences of PCR unmethylated (*I*) and methylated products (*II*) were compared. In unmethylated samples (*I*), all cytosines were changed to thymines (*asterisks*). In methylated samples (*II*), cytosines in CpG dinucleotide remained cytosines (*arrows*)
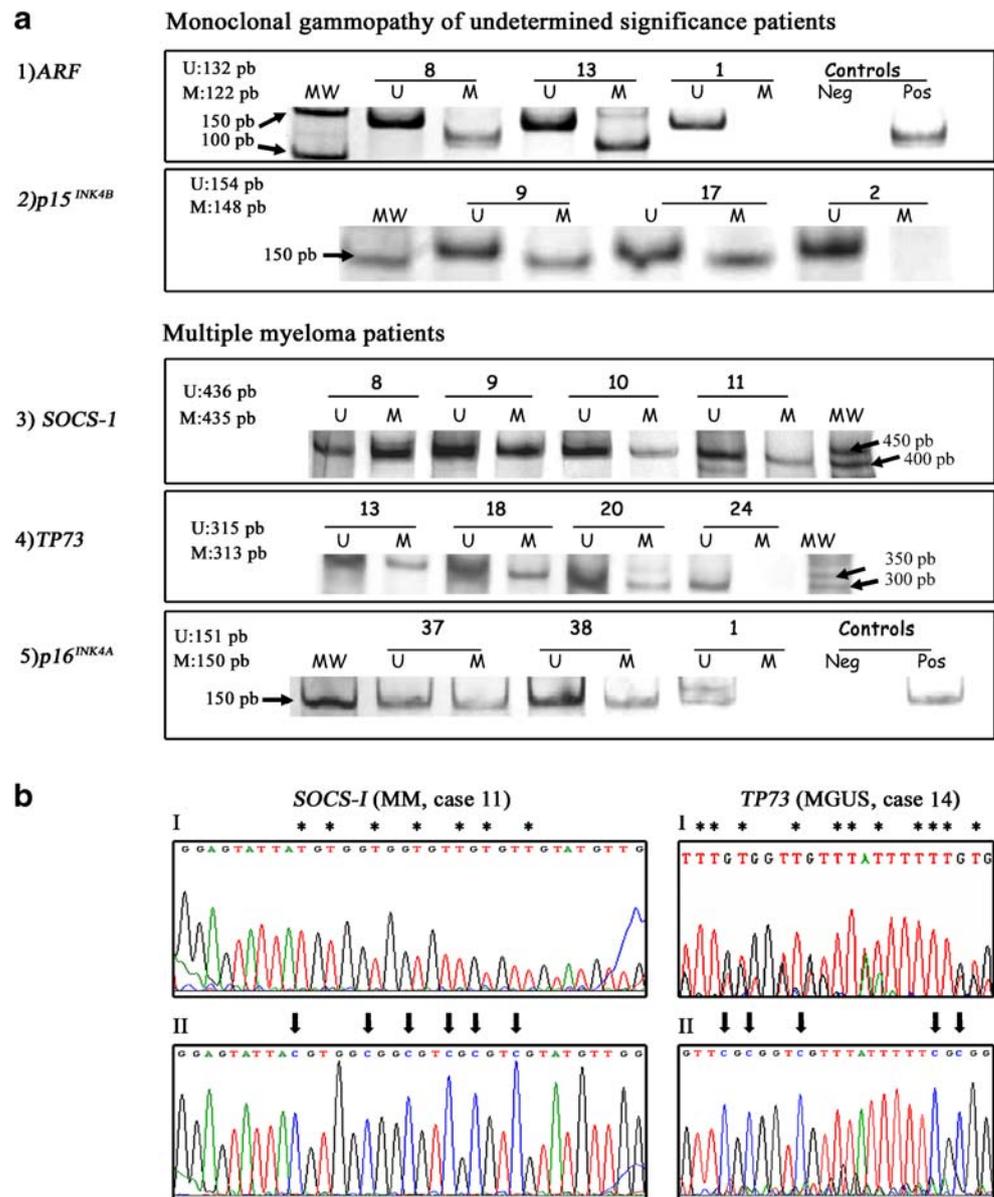

patients, mean age was significantly different between patients with methylated (82.3 year) and unmethylated (65.6 year) SOCS-1 gene ($p<0.001$; Supplementary material Table S1; Fig. 3). In MM, methylation of $p15^{INK4B}$ gene was more frequent in males ($p=0.009$) and in the IgG isotype ($p=0.038$), while the IgA isotype was only present in patients with unmethylated $p15^{INK4B}$ gene (Fig. 4). Survival analysis did not show differences in both OS and PFS between MM patients with or without methylation ($p= 0.78$ and $p=0.98$, respectively). RASSF1A and $p16^{INK4A}$ genes were not included in this evaluation due to the low number of cases with aberrant methylation. Data from ARF, SOCS-1, and TP73 are shown in Supplementary material

(Table S2). There were no correlations between clinico-pathologic characteristics and the total number of TSG methylated or the MI (Supplementary material, Table S3).

Discussion

There is strong evidence that DNA methylation plays a major role in silencing critical genes in cancer. In this study, we have evaluated methylation status of different TSG in MGUS patients and compared it with that of MM, by means of MSP followed by sequencing of the resulting product. MSP is a rapid and sensitive technique, currently

Fig. 3 Distribution of MGUS patients with methylated and unmethylated *SOCS-1* gene according to age. *M* methylated; *U* unmethylated. * *Significant differences with respect to unmethylated cases, p<0.001*
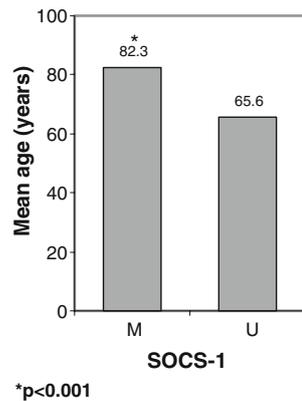
considered the standard for studying the methylation status of CpG islands in human cancer, especially if it is followed by the sequencing to confirm the identity of the methylated and unmethylated PCR products [16, 34, 43].

Our data showed that epigenetic events are frequent in MGUS and MM. However, the extension of methylation, measured by the MI, was different between both entities, being MGUS significantly less methylated than MM. Nevertheless, this relatively high frequency of methylation in MGUS would be consistent with it being a premalignant condition. In agreement with our data, a previous report by Takahashi et al. [46] found no differences in the MI of MM from that of lymphoma or leukemias, but it was higher than that of MGUS. In addition, we did not find correlation between clinicopathologic features and MI, indicating that the extent of methylation would not be a prognostic factor for patients with plasma cell disorders.

SOCS-1 gene methylation was significantly more frequent in MM than in MGUS patients, suggesting the methylation of this gene as an important step in the clonal evolution from MGUS to MM. The only previous report [42] about *SOCS-1* methylation studying MGUS and MM patients found comparable frequencies in both entities. Differences with our results could probably be explained by distinct clinicopathologic characteristics of MGUS patients

included in both series, but the absence of these data in the report of Reddy et al. [42] makes this comparison impossible to do. *SOCS-1* is an intracellular negative-feedback molecule that inhibits JAK-signal transducer and activator of transcription activation initiated by various stimuli, including IL-6. IL-6 is an essential growth and survival factor for tumor cells in MM [25]. In addition, it has been hypothesized that the dysregulation of cytokines might be involved in the transition from MGUS to MM [31, 39]. Thus, silencing of *SOCS-1* by methylation may allow uncontrolled proliferation of plasma cells, promoting disease progression from MGUS toward MM.

In MGUS patients, *ARF* and *p15*^{INK4B} genes showed similar methylation frequencies. For *ARF*, we found a higher methylation frequency than those observed in the literature [33, 44]. While for *p15*^{INK4B}, our values were in the range of those previously reported [19, 33, 44]. Regarding *p16*^{INK4A}, the frequency of 5% observed in our series was comparable with the lowest results previously published [5, 35, 47]. In our series, methylation of *p15*^{INK4B} was more frequent than that of *p16*^{INK4A} in agreement with those observed by Guillerm et al. [19]. In reference to *TP73* gene, the literature showed only one report of *TP73* methylation in MGUS patients [44], describing a comparable frequency (21%) to those observed in our series (33%). *TP73*, *p16*^{INK4A}, *p15*^{INK4B}, and *ARF* genes showed similar methylation frequencies in both MGUS and MM, suggesting that they are early events in the pathogenesis and development of plasma cell disorders and probably not involved in the progression from MGUS to MM.

In both entities, concurrent hypermethylation of *p15*^{INK4B} and *TP73* was observed. This suggests that disruption of pRb/CDK4/6 pathway by *p15*^{INK4B} methylation and the prevention of apoptosis by *TP73* methylation may collaborate with development of plasma cell disorders. Although the present association was not previously reported, concomitant methylation of *p16*^{INK4A} and *p15*^{INK4B} was found in 56% MM and 100% of plasmocytomas [38], and concurrent methylation of *p16*^{INK4A},

Fig. 4 Distribution of MM patients with methylated and unmethylated *p15*^{INK4B} gene according to sex and isotype. *M* males; *F* females. * *Significant differences with respect to unmethylated cases, p=0.009. † Significant differences with respect to unmethylated cases, p=0.038*
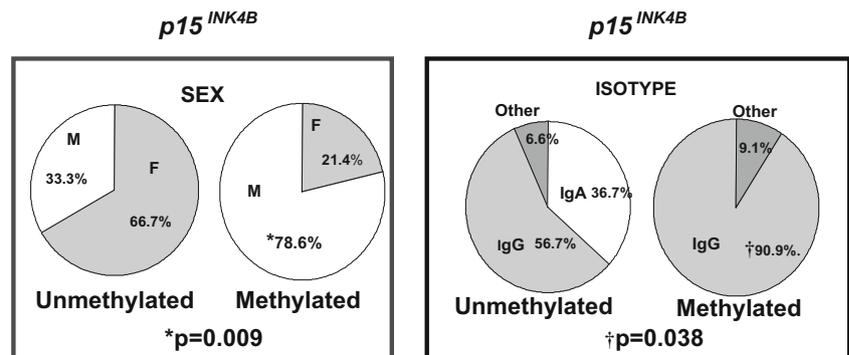
E-cadherin, and Death-associated protein kinase were found in connection with plasma cell leukemia development [15].

None MGUS patient had methylation of *RASSF1A* and *p27^{KIP1}* gene. For *RASSF1A*, our results are discordant to the only description in the literature [44] that found a frequency of 14% for this pathology. In reference to *p27^{KIP1}* gene methylation, there is no information about MGUS; meanwhile, Chim et al. [4], like us, did not find methylation of this gene in MM patients. Simultaneously, Nakatsuka et al. [37] found 25% of non-Hodgkin lymphoma patients with methylation of this gene, but it was scarcely affected in other hematologic malignancies.

The correlation with clinicopathologic parameters in MGUS patients showed significant difference in mean age between patients with methylated or unmethylated *SOCS-1* gene. Different data suggest that overall epigenetic modification is age-dependent. Wong et al. [50] found that frequencies of *p15^{INK4B}* and *p16^{INK4A}* methylation were higher in adult acute lymphoblastic leukemia than in children. In gastrointestinal tract, methylation begins in the normal colonic mucosa as an age-related event and progresses to hypermethylation in cancer [1]. Similar data were observed in prostate tissue. Although there are increasing methylation frequencies for *RARβ2*, *RASSF1A*, *GSTP1*, and *ESR1* genes in normal prostate tissue with age, their methylation frequencies were higher in prostate cancer than in normal cells [28].

The analysis of clinical data in MM showed an association of *p15^{INK4B}* methylation status with sex and IgG isotype, correlations that were not previously observed in other series. In addition, differences in OS and PFS between MM patients with and without hypermethylation were not statistically significant. In MM, some reports [15, 20, 35] found that OS was significantly lower in patients with methylated *p16^{INK4A}* gene, while others [16, 26, 44] showed no differences between methylated and unmethylated patients. Depil et al. [8] found that *SOCS-1* gene methylation does not seem to influence the clinical outcome. In addition, although Seidl et al. [44] did not find statistically significant OS between MM patients with and without *E-cadherin* methylation, they found an association between *E-cadherin* methylation and poor prognostic markers.

Concluding, our results show a similar pattern of methylation of *TP73*, *ARF*, *p15^{INK4B}*, and *p16^{INK4A}* in MGUS and MM, with a concurrent methylation of *p15^{INK4B}* and *TP73*, supporting MGUS as a premalignant condition. Interestingly, *SOCS-1* gene methylation was significantly more frequent in MM than MGUS, suggesting that methylation of this gene might be involved in the evolution from MGUS to MM. Silencing of *SOCS-1* may result in greater responsiveness to cytokines, which may favor neoplastic development.

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