Carcinogenesis vol.30 no.8 pp.1330–1335, 2009
doi:10.1093/carcin/bgp149
Advance Access publication June 16, 2009

Differential repetitive DNA methylation in multiple myeloma molecular subgroups

Valentina Bollati, Sonia Fabris[1], Valeria Pegoraro, Domenica Ronchetti[1], Laura Mosca[1], Giorgio Lambertenghi Deliliers[1], Valeria Motta, Pier Alberto Bertazzi, Andrea Baccarelli and Antonino Neri[1,*]

Center of Molecular and Genetic Epidemiology, EPOCA, Epidemiology Research Center, Università degli Studi di Milano and Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, 20122 Milan, Italy and [1]Leukemia Study Center, Department of Medical Sciences, Università degli Studi di Milano and Hematology 1-CTMO, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, 20122 Milan, Italy

*To whom correspondence should be addressed. Department of Medical Sciences, University of Milan, Via F. Sforza 35, 20122 Milano, Italy. Tel: +39 02 55033328; Fax: +39 02 50320403; Email: antonino.neri@unimi.it

Multiple myeloma (MM) is characterized by a wide spectrum of genetic changes. Global hypomethylation of repetitive genomic sequences such as long interspersed nuclear element 1 (LINE-1), Alu and satellite alpha (SAT-α) sequences has been associated with chromosomal instability in cancer. Methylation status of repetitive elements in MM has never been investigated. In the present study, we used a quantitative bisulfite-polymerase chain reaction pyrosequencing method to evaluate the methylation patterns of LINE-1, Alu and SAT-α in 23 human myeloma cell lines (HMCLs) and purified bone marrow plasma cells from 53 newly diagnosed MM patients representative of different molecular subtypes, 7 plasma cell leukemias (PCLs) and 11 healthy controls. MMs showed a decrease of Alu [median: 21.1 %5-methylated cytosine (%5mC)], LINE-1 (70.0%5mC) and SAT-α (77.9%5mC) methylation levels compared with controls (25.2, 79.5and 89.5%5mC, respectively). Methylation levels were lower in PCLs and HMCLs compared with MMs (16.7 and 14.8%5mC for Alu, 45.5 and 42.4%5mC for LINE-1 and 33.3 and 43.3%5mC for SAT-α, respectively). Notably, LINE-1 and SAT-α methylation was significantly lower in the non-hyperdiploid versus hyperdiploid MMs ($P = 0.01$ and 0.02, respectively), whereas Alu and SAT-α methylation was significantly lower in MMs with t(4;14) ($P = 0.02$ and 0.004, respectively). Finally, we correlated methylation patterns with DNA methyltransferases (*DNMTs*) messenger RNA levels showing in particular a progressive and significant increase of *DNMT1* expression from controls to MMs, PCLs and HMCLs ($P < 0.001$). Our results indicate that global hypomethylation of repetitive elements is significantly associated with tumor progression in MM and may contribute toward a more extensive stratification of the disease.

Introduction

Multiple myeloma (MM) is a neoplastic proliferation of monoclonal plasma cells (PCs) that can develop as a multistep process (1). Clinically, the disease may progress through monoclonal gammopathy of undetermined significance, smoldering myeloma, MM and plasma cell leukemia (PCL) (2). A wide spectrum of genetic changes have been observed in MM, including chromosomal translocations involving the immunoglobulin heavy chain locus at 14q32 and various partner genes (such as D-type cyclins, fibroblast growth factor receptor 3 and *MAF*), which are thought to play a critical role in transformation and disease progression (1,3–5); however, the molecular mechanisms underlying genetic instability are poorly understood. Epigenetics relate to stable and heritable patterns of gene expression and genomic functions that do not involve changes in DNA sequence (6). In mammals, DNA methylation, the most investigated epigenetic hallmark, is a reversible mechanism that modifies genome function and chromosomal stability through the addition of methyl groups to cytosine to form 5 methylcytosine (5mC). Cancer cells are characterized by hypermethylation of CpG islands and global genomic hypomethylation (7). Genomic DNA hypomethylation is likely to result from demethylation in repetitive elements, which account for ∼55% of the human genome and determine gene regulation and genomic stability (8). More than 90% of genomic 5 mCs lies within CpG islands located in transposable repetitive elements, including Alu, long interspersed nuclear element 1 (LINE-1) and satellite alpha (SAT-α) sequences that, due to their high occurrence throughout the genome, have been shown to correlate with global genomic DNA methylation content (9,10). The enzymes responsible for CpG methylation are the DNA methyltransferases (*DNMTs*), including *DNMT1*, *DNMT3a* and *DNMT3b* (11,12). It is thought that *DNMTs* may function either in the maintenance of DNA methylation, where methylated CpG sites on one DNA strand are copied, or in the *de novo* methylation, where both strands are initially unmethylated and methylation at novel sites is introduced (13).

In the past decade, a number of complex and interdependent epigenetic modifications have been identified that may contribute, along with genetic alterations, to cancer development and progression (14). However, the role of global DNA hypomethylation of repetitive elements in MM has never been investigated, and no data are available on the correlation between hypomethylation and different clinical and cytogenetic MM subgroups.

The purpose of the present study was the evaluation of Alu, LINE-1 and SAT-α methylation changes across different clinical and cytogenetic MM subgroups by means of a quantitative approach. In particular, we estimated global DNA methylation in LINE-1 and Alu elements and centromeric SAT-α sequences in a panel of 53 MM patients, 7 PCL patients and 11 healthy donors. In addition, 23 human myeloma cell lines (HMCLs) established from highly aggressive leukemic forms (2) were also investigated. Global DNA methylation was evaluated in the context of disease progression, presence of distinct chromosomal abnormalities and DNMTs expression levels.

Abbreviations: DNMT, DNA methyltransferase; HMCL, human myeloma cell line; LINE-1, long interspersed nuclear element 1; PC, plasma cell; PCL, plasma cell leukemia; 5mC, 5 methylcytosine; MM, multiple myeloma; PCR, polymerase chain reaction; SAT-α, satellite alpha.

Materials and methods

Patients, HMCLs and sample preparation

High-molecular weight DNA was isolated using standard protocols from CD138+ purified PCs from 53 MM and 7 PCL patients consecutively admitted to our hematology service and collected during standard diagnostic procedures. Informed consent was provided according to institutional guidelines. Genomic DNA from CD138+ purified PCs from 11 bone marrow aspirates of healthy donors was used as a negative control. The CD138+ selection was performed using immunomagnetic microbeads (MidiMACS system, Miltenyi Biotec, Auburn, CA) separation as described previously (15,16). The purity of the selected PCs population was assessed by means of morphology and flow cytometry and was ≥90% in all cases. Diagnosis and stage were determined according to the criteria by Durie *et al.* (17). The main clinical characteristics of the patient cohort are summarized in Table I.

DNA methylation changes were also investigated in a representative panel of 23 well-characterized HMCLs: U266, KMM-1, KMS-11, NCI-H929, JJN3, KMS-18, KMS-12, KMS-26, KMS-34, KMS-27, KMS-20, CMA-01, KMS-28, CMA-03, LP1, AMO1, RPMI-8226, CMA-02, OPM2, SK-MM-1, KM4, FR4 and NCU-MM-1 (2). The whole panel of patients, HMCLs and 4 of 11 healthy donors, were previously profiled with the GeneChip® Human Genome U133A (HG-U133A) arrays and publicly available on the Gene Expression Omnibus (GEO) website (http://www.ncbi.nml.nih.gov/geo) via GEO Series accession number GSE6205 and GSE13591 (18).

DNA extraction and bisulfite treatment of the DNA

DNA was extracted by a commercial kit (Promega, Madison, WI). In total, 1 μg DNA (concentration 50 ng/μl) was treated using EZ DNA Methylation-Gold™

Kit (Zymo Research, Orange, CA) according to the manufacturer's protocol. Final elution was performed with 30 µl of M-Elution Buffer. Bisulfite-treated DNA was stored at −20°C and used shortly after treatment.

Repetitive element polymerase chain reaction and pyrosequencing

DNA methylation was quantitated using bisulfite-polymerase chain reaction (PCR) and pyrosequencing (9). In brief, the samples were bisulfite treated and PCR amplified. The PCR primers were designed toward a consensus Alu or LINE-1 sequence and allowed the amplification of a representative pool of repetitive elements to serve as a surrogate for global DNA methylation changes. Analysis of DNA methylation in Alu and LINE-1 repetitive element was performed using previously published methods (9,19), whereas SAT-α analysis was settled as following. For each reaction, a 50 µl PCR was carried out in 50 µl of GoTaq Green Master mix (Promega), 1 pmol of the forward primer, 1 pmol of the reverse primer, 50 ng of bisulfite-treated genomic DNA and water. Bisulfite-modified DNA was amplified and genotyped with primers as follows: forward 5′-biotin-TTTTTATTAAAAATATAAAAATT-3′, reverse 5′-CCCAAACTAAAATACAATAA-3′ and sequencing primer 5′-AATAAC-TAAAATTACAAAC-3′; forward 5′-TTTTGAGTTAGGTGTGGGATATA-3′, reverse 5′-biotin-AAAATCAAAAAATTCCCTTTC-3′ and sequencing primer 5′-AGTTAGGTGTGGGATATAGT-3′; forward 5′-biotin-TGTAAG TGGATATTTGGATTATTGG-3′, reverse 5′-TTTCCAAAAAAATCTT-CAAAAAAAT-3′ and sequencing primer 5′-CTCAAAAATTTCTAAAAATA CTTCTC-3′ for Alu, LINE-1 and SAT-α, respectively. PCR conditions consisted of 96°C for 90 s, followed by 43°C for 60 s and 72°C for 120 s (45 cycles) for Alu; 95°C for 30 s, followed by 50°C for 30 s and 72°C for 30 s (45 cycles) for LINE-1 and 95°C for 60 s, followed by 55°C for 60 s 72°C for 60 s (45 cycles) for SAT-α. The size of bisulfite PCR products was 168 bp for LINE-1, 148 bp for Alu and 223 bp for SAT-α.

One of the primers, based on SAT-α sequence, was biotin-labeled and used to purify the final PCR product using Sepharose beads. The PCR product was bound to Streptavidin Sepharose HP (Amersham Biosciences, Uppsala, Sweden) and the Sepharose beads containing the immobilized PCR product were purified, washed, denatured using a 0.2 M NaOH solution and washed again using the Pyrosequencing Vacuum Prep Tool (Pyrosequencing, Westborough, MA), as recommended by the manufacturer. Then, 0.3 µM pyrosequencing primer was annealed to the purified single-stranded PCR product and pyrosequencing was performed using the PSQ HS 96 Pyrosequencing System (Pyrosequencing). Methylation quantification was performed using the provided software. The degree of methylation was expressed as %5-methylated cytosines (%5mC) over the sum of methylated and unmethylated cytosines. Prior to performing any DNA sequence analysis, the efficiency of sodium bisulfite conversion was assessed using a specific protocol that includes, for quality control purposes, a cytosine–thymine control. Because repetitive elements are often mutated and the assay is not able to distinguish the origin of TpG from either mutation of 5mC or conversion of an unmethylated cytosine, they do not represent a good target to assess the bisulfite conversion efficiency. For each sample, we tested the completeness of bisulfite treatment measuring a non-CpG cytosine contained in the highly conserved promoter of p15 gene. Given the high standardization of bisulfite treatment, the efficiency of bisulfite treatment measured in this position was >98% in all the samples. Every sample was tested three times for each marker to confirm reproducibility of our results. The average of the triplicates was used in the statistical analysis.

Molecular characterization of MM and PCL patients

The 53 MM and 7 PCL patients were investigated by fluorescence *in situ* hybridization for the main chromosomal alterations described in MM, including the most common *IGH* translocations, as well as structural aberrations represented by 17p13.1 and 13q14.3 deletions, 1q21 gain and ploidy status (see Table II). The fluorescence *in situ* hybridization procedure and specific probes for chromosome aberrations detection have been described previously (15,18,20,21).

Table I. Patients characteristics

	MM	PCL
Number of patients	53	7
Gender (*n*)		
Male	32	5
Female	21	2
Age (years)		
Median	67	72
Range	43–85	52–78
Paraprotein subtype (*n*)		
IgG	37	4
IgA	11	1
Light chain only	5	2
Durie–Salmon stage (*n*)		
I	19	—
II	18	—
III	16	—
Median percent of bone marrow PCs	18 (2–82)	80 (40–99)

Table II. DNA methylation levels in MM samples related to molecular cytogenetic characteristics

	ALU			LINE-1			SAT-α		
	n	Median (IQR[a])	*P*[b]	*n*	Median (IQR[a])	*P*[b]	*n*	Median (IQR[a])	*P*[b]
HD	28	21.8 (19.6–22.8)	0.12	28	72.1 (66.8–74.7)	**0.01**	27	80.6 (73.4–84.3)	**0.02**
NHD	24	20.1 (18.1–22.2)		24	60.5 (45.6–73.2)		23	67.4 (56.6–79.0)	
del(13)									
−	31	21.0 (18.3–22.6)	0.94	31	71.8 (59.1–74.5)	0.41	29	80.2 (69.2–84.2)	0.10
+	22	21.4 (18.2–22.5)		22	63.4 (60.0–73.2)		22	69.1 (61.7–82.9)	
del(17)									
−	48	20.9 (18.2–22.5)	0.80	48	69.3 (56.0–73.5)	0.36	46	77.9 (63.3–84.0)	0.20
+	4	20.6 (17.4–23.3)		4	72.0 (66.7–74.8)		4	80.5 (72.4–89.1)	
gain 1q21/1q42									
−	25	21.5 (18.9–23.1)	0.19	25	71.5 (63.2–74.7)	0.17	24	81.2 (70.6–84.2)	0.07
+	28	20.6 (18.0–22.3)		28	65.2 (54.2–73.2)		27	73.7 (61.7–80.6)	
t(11;14)									
−	43	21.3 (18.2–22.5)	0.71	43	70.6 (60.5–73.9)	0.22	41	78.9 (65.4–84.1)	0.80
+	10	19.8 (18.1–22.6)		10	60.1 (41.5–73.8)		10	70.7 (43.0–86.9)	
t(4;14)									
−	46	21.7 (18.6–22.8)	**0.02**	46	71.1 (60.4–73.3)	0.12	44	79.5 (67.4–84.2)	**0.004**
+	7	18.2 (15.0–21.0)		7	60.6 (52.6–63.2)		7	60.9 (38.7–66.7)	
MAF									
−	50	21.3 (18.2–22.6)	0.73	50	71.0 (60.4–74.3)	0.13	48	79.0 (65.4–84.2)	0.09
+	3	20.6 (16.4–22.9)		3	60.0 (43.8–63.6)		3	62.5 (56.6–65.5)	

Significant *P*-values are shown in bold. HD, hyperdiploid MMs; NHD, non-hyperdiploid MMs.
[a]Interquartile range (IQR): 25th and 75th percentiles are shown.
[b]Wilcoxon rank-sum test for difference between categories.

Statistical analysis

Spearman's rank correlation coefficients were used to assess associations among different DNA methylation variables. We assessed differences in DNA methylation and *DNMTs* expression among MMs, PCLs, HMCLs and healthy subjects using Kruskal–Wallis rank tests. This test was also used to evaluate differences in DNA methylation levels in MM samples according to monoclonal component and bone lesions.

We evaluated the association of DNA methylation levels with distinct molecular MM subgroups using Wilcoxon rank-sum tests. Two-sided *P*-values <0.05 were considered statistically significant. All analyses were performed in Stata/SE, version 10.0 (Stata Corporation, College Station, TX).

Results

Methylation levels in healthy subjects, MMs, PCLs and HMCLs

MMs showed a decrease of Alu (median: 21.1%5mC) and LINE-1 (70.0%5mC) methylation levels compared with controls (25.2 and 79.5%5mC, respectively). Accordingly, SAT-α DNA in MMs (77.9%5mC) displayed methylation levels lower than controls (89.5%5mC). Methylation in PCLs and HMCLs was lower than in MMs (16.7 and 14.8 versus 21.1%5mC for Alu; 45.5 and 42.4 versus 70.0%5mC for LINE-1; 33.3 and 43.3 versus 77.9%5mC for SAT-α DNA, respectively) (*P* for differences among categories and *P* for trend both <0.001; Figure 1).

Correlation between chromosomal abnormalities and DNA methylation

Global DNA methylation was evaluated in the context of different MM cytogenetics subgroups. Particularly, non-hyperdiploid tumors showed a significantly lower methylation in LINE-1 and SAT-α compared with hyperdiploid tumors (60.5 versus 72.1%5mC, $P = 0.01$ and 67.4 versus 80.6%5mC, $P = 0.02$, respectively). No statistically significant association between the ploidy status and Alu was observed (Table II). Patients with t(4;14) showed a significant lower methylation than patients without this lesion for Alu (18.2 versus 21.7%5mC, $P = 0.02$) and SAT-α (60.9 versus 79.5%5mC, $P = 0.004$). The *MMSET* overexpression levels in each of these patients were not significantly correlated with the respective global DNA methylation levels (data not shown). Finally, no significant associations of DNA methylation with del(13), del(17), gain 1q21/1q42 or translocation involving *MAF* genes were found (Table II).

Correlation between clinical characteristics and DNA methylation

Methylation status of LINE-1, Alu and SAT-α did not significantly vary in patients with the monoclonal components IgA, IgG or IgK. Furthermore, no significant associations between DNA methylation and the occurrence of bone lesions were found (data not shown).

DNMT1, DNMT3a and DNMT3b expression in healthy subjects, MMs, PCLs and HMCLs

To investigate the relationship between DNA hypomethylation and *DNMTs* expression, we evaluated the absolute expression levels of three distinct *DNMT* genes available for the whole panel of 53 MMs, 7 PCLs, 23 HMCLs and 4 healthy donors in our gene expression profiling database. As depicted in Figure 2, *DNMT1* showed an increasing median expression from controls (101.8) to MMs (143.3), PCLs (205.9) and HMCLs (1101) (*P* for difference among groups <0.001). *DNMT3a* showed a decreasing median expression from controls (60.5) to MMs (51.6) and PCLs (47.4), but an increased expression in HMCLs (81.9) (*P* for difference among groups <0.001). *DNMT3b* showed almost similar median expression levels among controls (37.4), MMs (35.0) and PCLs (39.4), but significant higher levels in HMCLs (271.5) (*P* for difference among groups <0.001; Table III).

Correlation between DNA methylation markers and DNMTs expression levels

As depicted in Table IV, we observed a strong correlation between the methylation levels of repetitive sequences Alu, LINE-1 and SAT-α. In addition, a moderate correlation was found between the expression levels of the three *DNMTs* we evaluated. Interestingly, we found that DNA methylation levels of LINE-1, Alu and SAT-α were inversely associated with the expression levels of *DNMT1*, *DNMT3a* and *DNMT3b.*

Discussion

In the present study, we investigated the methylation status of repetitive DNA elements in a panel of 11 healthy subjects, 53 MMs, 7 PCLs and 23 HMCLs to verify a possible correlation with different MM molecular subtypes, as well as with tumor progression. We found a progressive and significant methylation decrease in Alu, LINE-1 and SAT-α sequences from healthy control to MMs, PCLs and HMCLs, the latter showing the lowest methylation levels. Different methylation levels according to ploidy status and presence of t(4;14) translocation were also found. Finally, we evaluated whether the observed differences in DNA methylation might be correlated with the transcript levels of distinct DNMT genes previously generated on the same panel by microarray analysis.

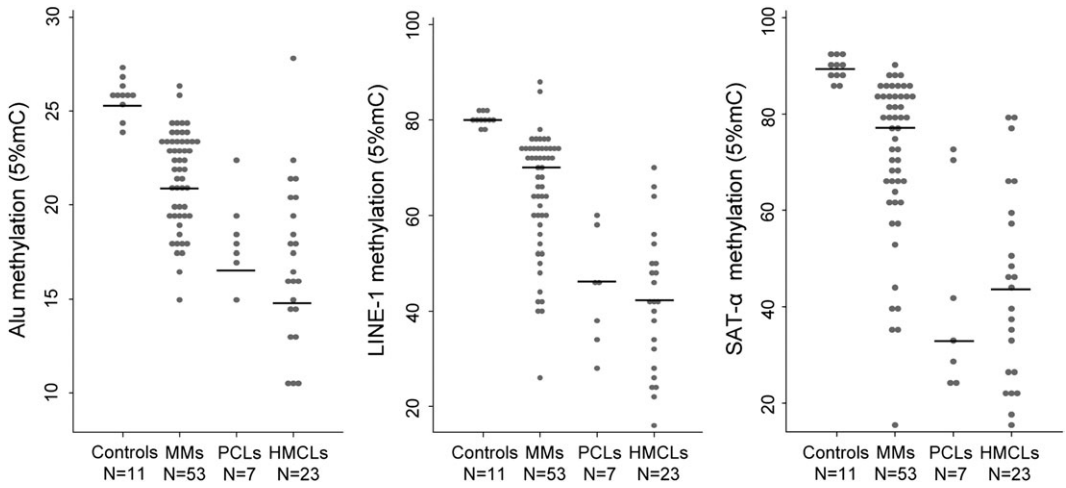

Fig. 1. Median methylation levels of Alu, LINE-1 and SAT-α repetitive genomic sequences in controls, MMs, PCLs and HMCLs. MMs showed a decrease of Alu, LINE-1 and SAT-α methylation levels compared with controls. Methylation levels of all repetitive elements were lower in PCLs and HMCLs compared with MMs ($P < 0.001$ for difference among groups and $P < 0.001$ for trend).

A quality of our study is that it was based on quantitative analysis of DNA methylation using pyrosequencing, which is highly reproducible and accurate at measuring small changes in DNA methylation (9,19). Each analysis was repeated in triplicate to minimize the assay variability and maximize statistical power.

Aberrant DNA methylation, including both global hypomethylation and gene-specific hypermethylation, has been frequently found in tumor cells (7). Global hypomethylation may result in chromosome instability and give selective advantage through activation of cellular genes that are usually silenced (22). DNA hypomethylation is expected to lead to the transcriptional activation of those repetitive sequences that still contain active promoters, as well as to alterations in genomic organization and stability (23). Several studies have suggested that mammalian DNA methylation may play an important role in maintaining genome stability and that DNA hypomethylation in cancer cells is related to genomic aberrations (24). A link between hypomethylation and the integrity of whole chromosome arms has been demonstrated in the human Immunodeficiency–Centromeric Instability–Facial Anomalies syndrome (25), in hypomethylation-induced T-cell lymphomas in mice (26) and in human colorectal (27), hepatocellular (28) and prostate carcinomas (23).

Genomic instability involving both numerical and structural chromosomal aberrations is a typical hallmark of MM (1,29,30), although the molecular mechanisms underlying these events are poorly understood. Previous investigations on MM have shown aberrant hypermethylation in several specific genes, including *p15*, *p16*, *E-CAD* and *DAPK* (5,31–38); however, the possible involvement of LINE-1, Alu and SAT-α hypomethylation in myeloma transformation and progression, unlike other different cancer types, has never been investigated.

Our study quantitatively determined that DNA hypomethylation is an important feature of MM and supported a possible link between global DNA hypomethylation and chromosomal instability in specific MM subtypes. We demonstrated a significant progressive reduction of the methylation of LINE-1, Alu and SAT-α in purified PCs across MM tumors with increasing levels of genomic instability, with the highest methylation in healthy subjects and decreasing levels in MMs and PCLs and the lowest levels in HMCLs. These findings are similar to

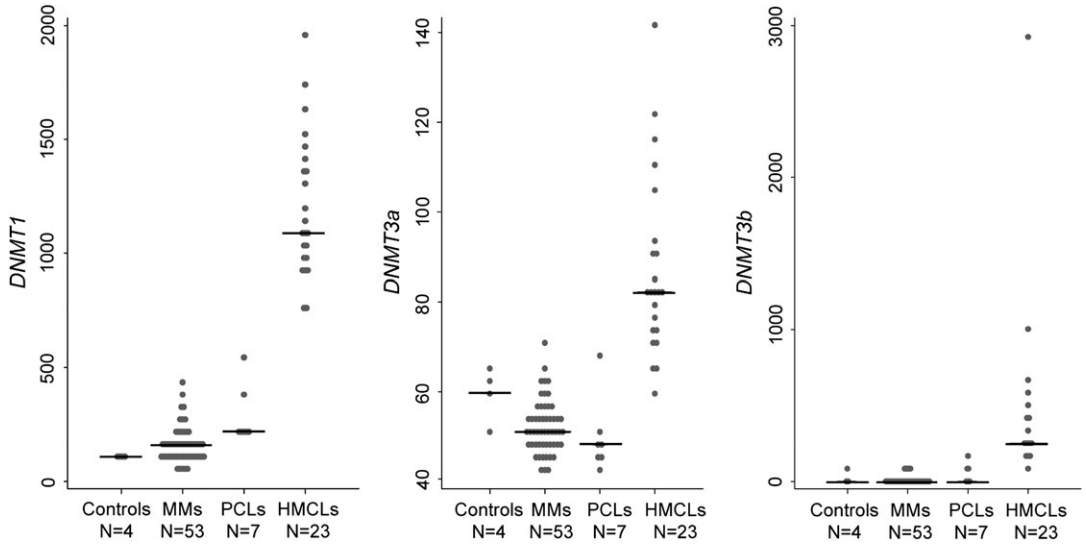

Fig. 2. The messenger RNA median expression levels of three DNMTs (*DNMT1*, *DNMT3a* and *DNMT3b*) in healthy subjects, MMs, PCLs and HMCLs, profiled by microarray analysis (see text). An increase from controls to MMs, PCLs and HMCLs was observed in the messenger RNA median expression levels of *DNMT1*. An increase in the messenger RNA median expression levels of *DNMT3a* and *DNMT3b* was found in HMCLs ($P < 0.001$ for difference among groups).

Table III. DNMTs expression in healthy subjects, MMs, PCLs and HMCLs

DNMT	Healthy donors Median (IQR[a])	MMs Median (IQR[a])	PCLs Median (IQR[a])	HMCLs Median (IQR[a])
DNMT1	101.8 (90.98–111.12)	143.3 (102.23–189.33)	205.9 (193.98–386.99)	1101 (954.95–1413.33)
DNMT3a	60.5 (54.96–63.83)	51.6 (48.46–55.07)	47.4 (44.11–50.21)	81.9 (72.50–92.95)
DNMT3b	37.4 (32.90–41.53)	34.35 (31.38–38.43)	39.4 (31.41–111.21)	271.5 (208.54–451.54)

[a]Interquartile range (IQR): 25th and 75th percentiles are shown.

Table IV. Correlation between methylation status of the different methylation markers (Spearman's rank correlation coefficients and *P*-value)

	ALU	LINE-1	SAT-α	*DNMT1*	*DNMT3a*	*DNMT3b*
ALU	—					
LINE-1	0.84 (<0.001)	—				
SAT-α	0.73 (<0.001)	0.89 (<0.001)	—			
DNMT1	−0.43 (<0.001)	−0.54 (<0.001)	−0.48 (<0.001)	—		
DNMT3a	−0.29 (0.01)	−0.27 (0.01)	−0.24 (0.03)	0.44 (<0.001)	—	
DNMT3b	−0.41 (<0.001)	−0.46 (<0.001)	−0.42 (<0.001)	0.63 (<0.001)	0.56 (<0.001)	—

those reported in other types of cancer showing significant decreases in global genomic methylation levels associated with tumor progression (39). It has been suggested that DNA hypomethylation in cancer may facilitate illegitimate mitotic recombination leading to chromosomal breaks, chromosomal translocations and/or allelic loss (40). In particular, loss of methylation in the pericentromeric chromosome regions may lead to breakage of the regions themselves and high frequency of chromosome rearrangements/breakpoints has been observed in many tumors, including MM (41,42). The evidence of a significant hypomethylation of LINE-1 and SAT-α in the non-hyperdiploid MM subgroup and Alu and SAT-α in the t(4;14) MM tumors, known to be associated with a poor prognosis (43–47), supports the suggestion that hypomethylation of distinct classes of repetitive elements may contribute to the pathogenesis and progression of MM and may represent a useful marker for risk stratification.

Particularly interesting is the finding that the methylation levels of the three different markers of global methylation were highly correlated. This suggests a common mechanism of methylation maintenance of such elements in MM (48). In addition, this correlation is suggestive for a genome-wide hypomethylation rather than a random hypomethylation of individual repetitive elements followed by the selection for the affected cell, as reported in other tumors (39).

DNMTs are the major determinants of physiological DNA methylation levels (49), but their role in cancer is uncertain (10). We identified a progressive and significant increase in *DNMT1* expression from healthy subjects to MMs, PCLs and HMCLs ($P < 0.001$). This finding, associated with global DNA hypomethylation, may appear paradoxical. Since *DNMT1* is responsible for copying methylation patterns after DNA synthesis, one possible explanation is that elevated *DNMT1* expression levels may reflect the progressive increase of the proliferative activity in the different categories rather than its involvement in DNA hypomethylation. This conclusion is consistent with previous studies on different types of human cancer where *DNMT1* has been reported to be proliferation dependent (7,50). *DNMT3a* showed a moderate downregulation in MMs and PCLs compared with controls but a significant overexpression ($P < 0.001$) in HMCLs. *DNMT3b* appeared to be overexpressed at very high levels in HMCLs. Further studies investigating gene-specific DNA methylation may help to explain the different patterns of *DNMT3a* and *3b* expression in distinct types of PC dyscrasias. Overall, these findings indicate that aberrant expression of *DNMTs* is not probably the cause of hypomethylation pattern suggesting that other mechanisms may be involved in determining genome-wide hypomethylation in MM.

In conclusion, our data suggest that epigenetic changes observed using a quantitative method may lead to a better understanding of MM pathogenesis and may contribute to identify novel markers useful to assess risk stratification and disease progression.

Funding

CARIPLO Foundation (2007-5469); Associazione Italiana Ricerca sul Cancro to A.N.; Associazione Italiana Leucemie, Milano.

Acknowledgements

Conflict of Interest Statement: None declared.

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Received April 2, 2009; revised June 3, 2009; accepted June 11, 2009