Thalidomide alters nuclear architecture without *ABCB1* gene modulation in drug-resistant myeloma cells

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Received March 18, 2009; Accepted May 7, 2009

DOI: 10.3892/ijo_00000376

Abstract. *ABCB1* gene overexpression has been described as an important mechanism for resistance to conventional chemotherapy in multiple myelomas. In the refractory multiple myelomas, other drug regimens have been successfully applied, including thalidomide treatments. Besides its well-documented anti-angiogenic effects, thalidomide therapy could result in a decrease in *ABCB1* gene expression. In this study, we analysed the effects of a 24-h short-term treatment by thalidomide or its active metabolite phthaloyl glutamic acid (PGA) on nuclear chromatin higher-order organisation and *ABCB1* gene expression in drug-sensitive and drug-resistant 8226 human myeloma cells. As compared to sensitive cells, 8226-Dox40 drug-resistant cells exhibited an increase in chromatin texture condensation and *ABCB1* gene overexpression. At this gene promoter level, the -50 and -100 GC boxes displayed an unmethylated profile in drug-sensitive cells whereas drug-resistant cell promoter GC boxes were fully methylated. Thalidomide and PGA induced significant chromatin textural changes in 8226-Dox40 drug-resistant cells only with neither alteration in *ABCB1* gene expression nor methylation profile of its promoter. Conversely thalidomide and PGA induced down-regulation of VEGF gene expression in both drug-sensitive and -resistant myeloma cells. These data suggest that short-term treatments by thalidomide or PGA do not induce any significant change on *ABCB1* gene expression though they modulate chromatin supra-organisation in drug-resistant 8226 human myeloma cells.

Introduction

Drug resistance is a major drawback for cancer chemotherapy treatments and previous studies have demonstrated that the overexpression of the *ABCB1* gene-encoded P-glycoprotein (P-gp) represents an important mechanism in multidrug-resistance development (1). Moreover, in cell lines derived from various tissues (lung, ovary, breast), we and others reported that this drug resistance was associated with alterations in nuclear architecture that could impact epigenetic *ABCB1* gene control (2-5).

ABCB1 gene overexpression has also been demonstrated in multiple myelomas (MM) resistant to conventional chemotherapy (6). In these refractory MM, other drug regimens have been successfully applied, including thalidomide or lenalidomide treatments. Thalidomide was previously used as a sedative drug before being withdrawn due to its teratogenic effects. However, more recently, it gained a renewed interest and is widely used in the treatment of various inflammatory and autoimmune disorders, as well as tumours, particularly MM (7,8).

Besides the well-documented anti-angiogenic effects of thalidomide by inhibition of VEGF and bFGF, several mechanisms have been proposed to explain the anti-myeloma activity of this molecule: immunomodulation of cytokine production as TNFα, IL-6 or IL-12, inhibition of cell adhesion by modulation of VCAM, ICAM-1 and CD31, and direct inhibition of tumour growth through inhibition of NF-κB (7,8). Moreover, it has been reported that thalidomide therapy could result in a decrease in *ABCB1* gene expression in a patient with primary resistant MM (9). The mechanisms underlying this decrease remain unclear but could include some epigenetic control of the *ABCB1* gene expression. We showed previously that inhibition of histone deacetylases led to *ABCB1* gene modulation, both in drug-sensitive and -resistant cells, a phenomenon associated with chromatin structure modifications (3,10,11). Considerable efforts have been made recently towards the definition of the regulatory mechanisms that control the expression of *ABCB1* gene in tumour cells (reviewed in ref. 12). The *ABCB1* promoter lacks a TATA-box. Like most TATA-less promoters, *ABCB1* promoter contains an inverted CCAAT box (Y-box) close to a -50 GC box where NF-Y, Sp1, and Sp3 factors can bind. Several other elements have also been described, particularly a -110 GC box able to bind a repressor factor (12). *ABCB1* promoter appears therefore as a GC-rich sequence and interestingly, thalidomide has been shown to down-regulate transcript levels of GC-rich promoter genes in MM (13).

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Key words: thalidomide, drug resistance, ABCB1, myeloma cell lines

The aim of this work was therefore to analyse the effects of a short-term treatment by thalidomide and its functional derivative phthaloyl glutamic acid PGA on *ABCB1* expression in drug-sensitive and resistant human myeloma cells.

Materials and methods

Chemicals. Thalidomide and phthaloyl glutamic acid (PGA) were purchased from Sigma-Aldrich (St. Quentin-Fallavier, France) and dissolved at a concentration of 0.125 M in DMSO. Doxorubicin was purchased from Sigma-Aldrich and dissolved at a concentration of 17 mM in water. These stock solutions were kept at -20˚C and dilution was performed in culture medium to be added at the appropriate concentration to cell culture. All other chemicals were obtained from standard sources.

Cells. The RPMI-8226 human multiple myeloma cell and its multidrug-resistant variant 8226-Dox40 (14) selected with doxorubicin were kindly provided by Professor W.S. Dalton, Tampa, FL. The two cell lines were grown in DMEM medium (Gibco-BRL, France) supplemented with glutamine and containing 10% fetal calf serum (FCS), 100 U/ml penicillin G, and 100 μg/ml streptomycin at 37˚C in a humidified 95% air, 5% CO_2 atmosphere. The resistant 8226-Dox40 cell line, which overexpresses *ABCB1* gene, was regularly submitted to 4.10^{-7} M of doxorubicin to ensure against revertants. Cells were maintained in drug-free medium for 1 week prior to experiments. These cells were about 10-fold more resistant to doxorubicin. All experiments were performed with cells in exponential growth phase. Cell viability was evaluated using Trypan-blue staining.

Image cytometry. Cells were cytocentrifuged on glass slides. The cell spots were air-dried and fixed in alcohol-formalin mixture (95% ethanol, 3% formaldehyde in saline: 3:1) for 10 min. After acid hydrolysis with 5 M HCl for 30 min, they were stained by the Feulgen method as previously described (11). Image cytometry was performed with an image analysis system (SAMBA 2005, Samba Technologies, Meylan, France) coupled to a color 3CCD camera (XC-007P, Sony Corp., Japan) and a microscope (Axioscop, Karl Zeiss, Oberkochen, Germany). Measurements were made with a plan-achromat x40 objective. At least 200 cells were analyzed on each microscopic slide and 2 separate experiments were performed. Thirteen parameters were computed from each nuclear image: three morphometric features (nuclear area NA, nuclear perimeter NP, and form factor FF), one densitometric (integrated optical density IOD), and nine textural parameters. The distribution of nuclei according to DNA-IOD was plotted to provide cell cycle distribution and the margins defining G_0G_1 and G_2 peaks were defined as modal IOD ± 15% as previously described (2). At least 100 reference cells (mouse hepatocytes) were measured in the same conditions for the calibration of the normal diploid (2c) value. Nine textural features were calculated on the nuclear image after reduction to 16 gray levels by linear rescaling. Four features were calculated on the gray-levels co-occurrence matrix : local mean of gray levels (LM), energy (E), entropy (ENT), and inertia (I). Five parameters were calculated on the run-length matrix: short run-length emphasis (SRE), long run-length emphasis (LRE), gray levels distribution (GLD), run-length distribution (RLD), and run-length percentage (RPC) (15).

The distribution, means, and SD of the nuclear parameters were calculated for each cell population. Differences between groups were analysed by discriminant analysis completed by a canonical analysis. This feature-reduction technique derives canonical variables (CVs), which are linear combinations of the independent quantitative variables. Usually, the first CV is the most important, in that it shows the highest variation among the groups. Significance of the differences between parameters and CV values was estimated by the Student's t-test after Bonferonni correction for multiple groups and variables.

RT-PCR analysis. Total RNAs were isolated using TRIzol® reagent (Invitrogen, Abingdon, UK) in accordance to the manufacturer's protocol. The amount of RNA was analysed by QuBit™ fluorimeter technology (Invitrogen) and the quality was verified by 2% agarose gel. One microgram of total RNA were reverse-transcribed using Moloney-Murine Leukemia Virus reverse transcriptase (M-MLV, Invitrogen) and random primers in a 20-μl final volume.

Multidrug resistance (*ABCB1*), vascular endothelial growth factor (VEGF), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) transcripts were analysed by semi-quantitative reverse-transcription polymerase-chain-reaction (RT-PCR) using the following gene-specific oligonucleotide primers (Proligo SAS, France; primers final concentration: 1 μM): *ABCB1* forward: 5'-CCCATCATTGCAATAGCAGG-3', reverse: 5'-GTTCAAACTTCTGCTCCTGA-3'; *VEGF* forward: 5'-GAAGTGGTGAAGTTCATGGATGTC-3', reverse: 5'-CGATCGTTCTGTATCAGTCTTTCC-3'; *GAPDH* forward: 5'-CTCTGCCCCCTCTGCTGATGC-3', reverse: 5'-CCATCACGCCACAGTTTCCCG-3'. The *ABCB1* and *GAPDH* cycling parameters consisted of an initial denaturation at 95˚C for 5 min, followed by 24 cycles of 94˚C for 30 sec, 59˚C for 30 sec, and 72˚C for 30 sec with a final extension at 72˚C for 10 min. The *VEGF* PCR conditions were as follows: an initial denaturation at 95˚C for 5 min, followed by 26 cycles of 94˚C for 45 sec, 54˚C for 1 min, and 72˚C for 2 min, with a final extension at 72˚C for 10 min.

Following PCR, samples were subjected to electrophoresis on 2% agarose gel and bands were visualized by UV transillumination using ethidium bromide staining prior to photography then analysed by densitometry. Results were expressed in arbitrary unit (a.u.).

DNA extraction and bisulfite sequencing analysis. Genomic DNA was extracted using genomic DNA extraction kit (Qiagen, Courtaboeuf, France) according to the manufacturer's protocol. DNA (2 μg) was subjected to sodium bisulfite treatment using EpiTect® Bisulfite Kit (Qiagen). After bisulfite conversion, DNA was cleaned up using spin column as described in EpiTect Bisulfite Kit. The *ABCB1* promoter 5'-flank sequence was analysed (GenBank accession no. L07624). The primer sequences used are, forward: 5'-GGTGATATAGAATTGGAGAGG-3', reverse: 5'-CCTCAAAAAACCCTTCTCC-3'. A 602-bp-DNA fragment corresponding to the region between the positions

Table I. Cell cycle characteristics of 8226 cell lines.

Cells	Treatment	DNA index[a]	% cells in cell cycle phase[a]			
			G_0/G_1	S	G_2	M
8226	None	1.81±0.02	56±3	9±2	29±2	6±1
	Thalidomide	1.77±0.01[b]	51±3	14±3	32±4	3±1
	PGA	1.76±0.03	46±7	16±4	35±4	3±1
8226-Dox40	None	1.79±0.05	43±6	18±2	34±6	5±1
	Thalidomide	1.64±0.04[b]	53±4	18±3	27±2	2±1
	PGA	1.57±0.06[b]	53±3	16±1	28±3	3±1

[a]Values are mean ± standard deviation from 3 separate measurements (100-200 cell nuclei per sample). [b]Significantly different from untreated cells (t-test).

-381 and +221, relative to the major transcription start site, containing 28 CpG dinucleotides was amplified. PCR amplification was performed from 0.5 to 2 μl of modified DNA in a reaction mix of 25 μl containing 1X buffer, 1X Qbuffer, 1 μM of each primers, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 2.5 U of HotStarTaq DNA Polymerase (Qiagen). The PCR conditions were: an initial denaturation at 95˚C for 15 min, followed by 40 cycles of 94˚C for 50 sec, 61˚C for 50 sec, and 72˚C for 1 min, with a final extension at 72˚C for 10 min. PCR products (10 μl) were visualized by 2% agarose gel electrophoresis with ethidium bromide staining before sequencing. After classical PCR sequencing, PCR products were subjected to DNA sequencing with ABI 3130 (Applied Biosystems, Foster City, CA) and analysed.

Acid extraction and immunoblotting of histones. Histone acid extraction and immunoblotting analysis were performed as described previously (10). Antibodies (Upstate Biotechnology, Lake Placid, NY) were used at the following dilutions: anti-acetylated histone H3 (1:20000), anti-acetylated histone H4 (1:2000), and peroxidase-conjugated anti-rabbit secondary antibody (1:2000).

Results

Cell viability and cell cycle. We examined the effects of 250 μM thalidomide and PGA on the viability of drug-sensitive 8226 and drug-resistant 8226-Dox 40 cells. In both cell lines, these drug concentrations did not affect significantly cell viability (data not shown). To investigate the effect of thalidomide and PGA on cell cycle progression, DNA content was measured by image cytometry (Table I). In both cell lines, 24 h of treatment with 250 μM of thalidomide induced a significant decrease in DNA-index. The same phenomenon could be observed with PGA in drug-resistant cells. Thalidomide and PGA did not modify significantly the percentage of cells in the various cycle phases in either cell line.

Analysis of nuclear phenotype. Values of the morphometric and textural features are listed in Table II. The nuclear area (NA) appeared smaller in 8226-Dox40 cell line correlating with a decreased nuclear perimeter (NP). 8226-Dox40 cells, as compared with 8226, showed an decreased FF, underlining a rounder and more regular nuclear aspect. They also displayed higher-order chromatin organization changes, as demonstrated by significant decreases in E and GLD, thus underlining a

Table II. Nuclear geometric and textural parameters values in drug-sensitive (8226) and drug-resistant (8226-Dox40) cell lines.

Parameters	Parameter value[a]		Correlation with nuclear area (R)
	8226 (n=607)	8226-Dox40 (n=299)	
NA	7.57±2.02	6.24±1.83[b]	-
NP	3.82±0.58	3.32±0.53[b]	0.869
FF	1.59±0.23	1.48±0.22[b]	-0.001
LM	2.70±0.71	3.59±0.82[b]	-0.137
E	7.29±2.65	5.21±1.80[b]	0.094
ENT	2.99±0.38	3.32±0.36[b]	-0.124
I	3.18±0.75	3.61±1.04	-0.149
SRE	6.16±0.38	6.36±0.42	-0.151
LRE	5.72±1.18	5.21±1.03	0.100
GLD	17.66±4.39	13.62±3.23[b]	0.122
RLD	3.65±0.41	3.86±0.46	-0.152
RPC	10.47±0.90	10.90±0.90	-0.128

[a]Values are mean ± standard deviation, and data are expressed in arbitrary units. E, energy; ENT, entropy; FF, form factor; GLD, gray level distribution; I, inertia; LM, local mean of gray levels; LRE, long run-length emphasis; NA, nuclear area; NP, nuclear perimeter; RLD, run-length distribution; RPC, run-length percentage; SRE, short run-length emphasis. [b]Significantly different from 8226 cells (t-test after Bonferroni correction for multiple variables).

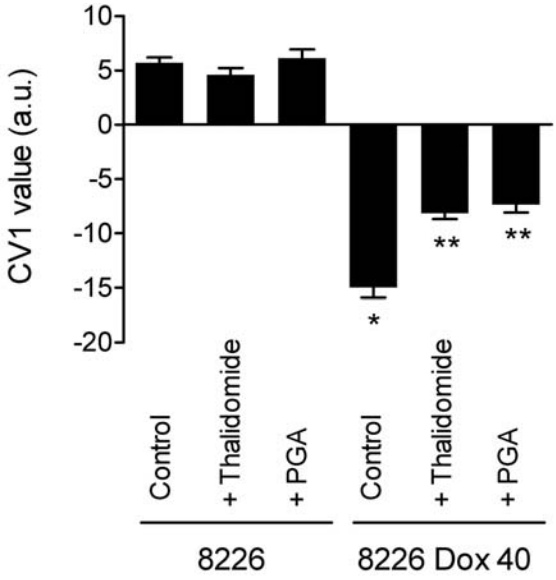

Figure 1. Analysis of nuclear texture in human myeloma 8226 and 8226-Dox40 cell lines treated or not for 24 h with 250 μM thalidomide or PGA. First canonical variable (CV1) values were expressed in arbitrary units (a.u.). *p<0.05 as compared to 8226 control values; **p<0.05 as compared to 8226-Dox40 control values (Student's t-test after Bonferroni correction).

different (more chaotic) distribution of chromatin that was confirmed by an increase in ENT, which suggested increased image contrast. LM, a feature that provides a global estimate of the whole chromatin condensation, appears also increased. Interestingly, all these features appear independent of the variations in nuclear area. These cell lines were therefore incubated with 250 μM of thalidomide and PGA for 24 h. The modifications of textural features were expressed as variations in the value of the 1st canonical variable (CV1) calculated after discriminant analysis and which explains 59% of the variance between the groups (Fig. 1). When 8226-Dox40 cells were treated with thalidomide and PGA, their chromatin texture showed significant alterations. The specific weight of the different computed parameters in the CV1 calculation is shown in Table III. The parameters have been ranked by weight absolute values and a minus sign implies a negative contribution to CV1 computation for the corresponding parameter. Analysis of Fig. 1 and Table III shows that thalidomide or PGA-treated drug-resistant cells displayed a chromatin decondensation (decrease of ENT, RPC, and I), with a less compact chromatin distribution (increase in E), and the appearance of a more homogeneous texture (increases in SRE, GLD, and LRE). On the contrary, drug-sensitive 8226 cells treated with thalidomide or PGA did not display chromatin changes as measured by image cytometry.

Nuclear histone acetylation and ABCB1 promoter methylation status. In order to investigate if the level of histone H3 and H4 acetylation could play some role in the chromatin structural changes observed in 8226-Dox40 drug-resistant cells, this expression was analysed by immunoblotting using anti-acetylated H3 and H4 antibodies in both cell lines. As shown on Fig. 2, thalidomide or PGA did not modulate these levels in either 8226 or 8226-Dox40 cells.

Table III. Specific weight values of textural parameters in the computation of the first canonical variable.

Parameter[a]	Specific weight in CV1 computation (a.u.)
E	78.27
ENT	10.25
RLD	9.38
LM	-4.91
LRE	-2.89
I	0.52

[a]Features were ranked according to their absolute weight value.

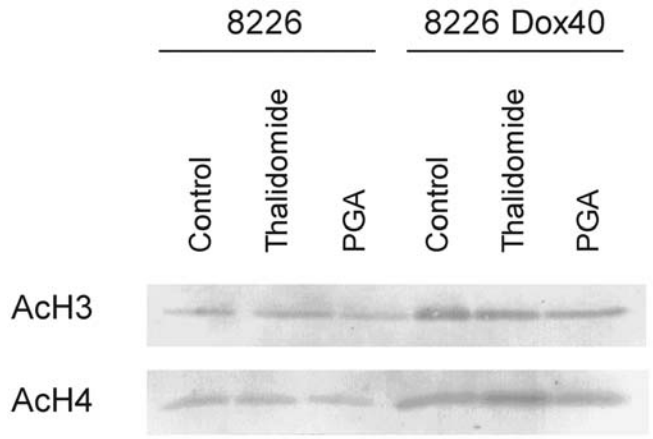

Figure 2. Thalidomide or PGA effects on histone H3 and H4 acetylation levels. Nuclear extracted histones from 8226 and 8226-Dox40 cells, both treated or not for 24 h with 250 μM thalidomide or PGA were resolved by SDS-PAGE, transferred to nitrocellulose membranes, and probed with specific anti-acetylated histone H3 and H4 monoclonal antibodies.

In parallel, the DNA methylation status was evaluated by bisulphite treatment followed by sequencing of a -381 to +221 sequence containing 28 methylation CpG sites which encompasses the -50 and -110 GC boxes of the *ABCB1* promoter in both cell lines. Observed data indicated that all cytosine residues analysed within this sequence were unmethylated in 8226 cells. On the contrary, the same sites displayed complete methylation in 8226-Dox40 cells. Further treatments with 250 μM thalidomide or PGA did not induce any change in these methylation profiles (Fig. 3).

ABCB1 and VEGF gene expressions. In order to evaluate a putative role of thalidomide in *ABCB1* gene regulation, the expression of this gene was explored by RT-PCR in 8226 and 8226-Dox40 cells treated or not with thalidomide or PGA for 24 h. The results of these analyses appear in Fig. 4. As expected, drug-resistant 8226-Dox40 cells strongly over-express *ABCB1* mRNA. Thalidomide or PGA treatments did not induce any significant *ABCB1* gene expression in 8226 cells (where basal expression could not be recorded), whereas gene expression was unaffected in 8226-Dox40 cells.

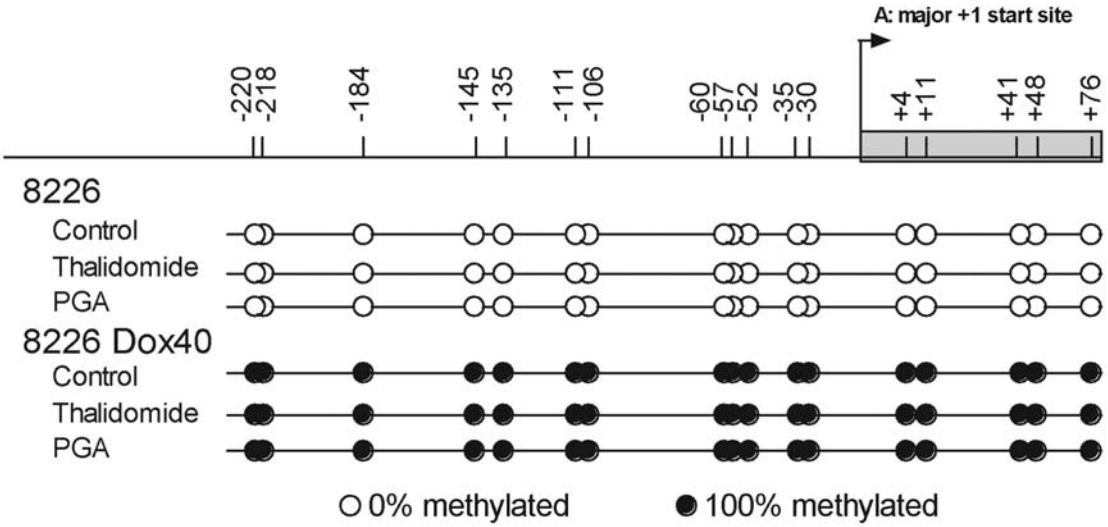

Figure 3. DNA methylation of the *ABCB1* promoter in 8226 and 8226-Dox40 cells treated or not with 250 μM thalidomide or PGA. The analysed sequence contains 28 methylation sites. For clarity, 17 only are displayed in the figure, with special emphasis on the -50 and -110 GC boxes. The 11 remaining sites displayed the same methylation profiles.

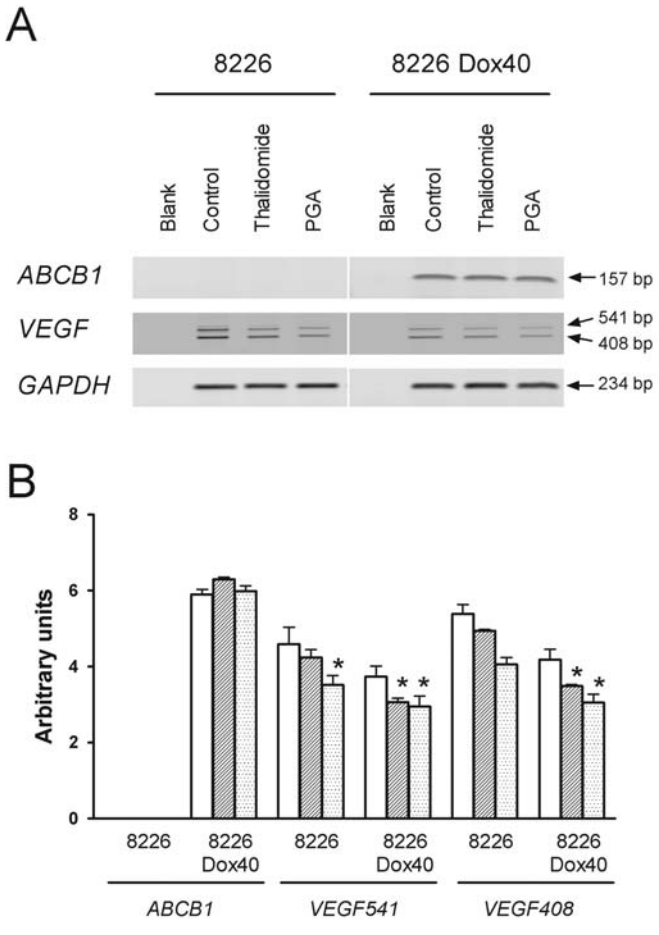

Figure 4. (A) Semi-quantitative RT-PCR assay for the *ABCB1* and *VEGF* genes in 8226 and 8226-Dox40 cells. Cells were treated or not for 24 h with 250 μM thalidomide or PGA. *GAPDH* was used as internal control. A representative experiment from a series of three is shown. (B) Graphic representation of the variations of the *ABCB1* and *VEGF*/*GAPDH* expression ratios. $^{*}p<0.05$ as compared to untreated control cells.

As it has been reported that thalidomide could modulate VEGF expression, *VEGF* gene was used as a positive control. PGA and to a lesser extent thalidomide induced a down-regulation of *VEGF* gene expression in both myeloma cell lines at the levels of the 408- and 451-bp amplicons (corresponding to 121 and 165 isoforms of VEGF-A). Such a down-regulation was not observed in the presence of 250 μM phthalimide, the inactive metabolite of thalidomide (data not shown).

Discussion

It has been proposed that chromatin remodeling is involved in the control of *ABCB1* gene expression. Considerable experimental evidence suggests that histone acetylation and DNA methylation are involved in this control (16-19). Consistent with these and other previous studies we also observed that treatments which modulate nuclear architecture could result in *ABCB1* up- or down-regulations in various tumour cell lines (3,10,11).

Thalidomide represents now a central component to most therapeutic strategies for the treatment of MM, particularly in the treatment of refractory disease. However, despite numerous studies, the mechanisms by which thalidomide exerts its anti-myeloma effects are not fully understood. It has been reported that this drug modulates the expression of adhesion molecules involved in cell-cell and cell-matrix adhesion and of numerous cytokines, particularly bFGF and VEGF, consequently inhibiting tumour angiogenesis. Thalidomide also regulates NF-κB complex activity, down-regulating the expression of genes implicated in angiogenesis and inflammatory response (7,8). In the present study we observed that a short-term thalidomide treatment induced chromatin texture alterations in myeloma cells.

Nuclear texture is a reflection of the overall structure of the nuclear chromatin (5,20). Studies of nuclear texture features have proved to be useful for the development of treatment protocols or to be effective as markers for cancer screening (21). However, the mechanisms that support modifications in nuclear texture are not fully understood. In the present study, we observed that 8226-Dox40 drug-resistant myeloma cells displayed significant chromatin alterations when

compared to their drug-sensitive counterparts. Drug-resistant cells exhibit a more condensed and irregular chromatin aspect, associated with an increase in LM and a decrease in GLD, two features giving a global estimate of the whole chromatin condensation. However, these variations in condensation are associated with modifications in chromatin spatial organisation. It has been proposed that chromatin texture could be described in terms of condensation (compactness of chromatin segments), distribution (relative proportions of compactness levels), and organisation (topographic arrangements of condensation degrees) (15). In 8226-Dox40 cells, significant variations were observed in E, and ENT, two features related to the spatial organization and distribution of the chromatin (15), and whose values are correlated neither to nuclear area nor to global chromatin condensation. Significant changes in chromatin higher-order organisation, evaluated through nuclear texture, have already been reported as markers of tumour cell aggressiveness or metastatic potential, either in experimental or clinical samples (22-25). For instance, LM displays significant increases in invasive bladder tumours as compared to superficial tumours (25) and appears linked (together with E and GLD) to tumour size in breast carcinomas (unpublished data).

Moreover, we reported previously similar chromatin changes in 8226 cells resistant to glucocorticoids, suggesting that the observed alterations could represent a drug (and mechanism)-independent marker of drug-resistance in these myeloma cells (26). However, these chromatin changes in drug-resistant cells can be modulated by thalidomide treatment. Thalidomide induces the appearance of a more homogeneous and decondensed chromatin in 8226-Dox40 cells but remains ineffective at this level on 8226 drug-sensitive cell nuclei. This effect can also be observed using the thalidomide active metabolite PGA.

Changes in chromatin higher-order organisation may reflect changes in the activation patterns of genes and epigenetic mechanisms may then play a significant role in the definition of texture phenotype. In this context, we reported previously that histone deacetylases inhibitor TSA could induce chromatin texture changes in tumour cell lines (H69, OV1) and that this effect could, in some cases, be associated with *ABCB1* gene expression regulations (10,11). Previous studies indicate that thalidomide could also play a role on drug-resistance. Thalidomide significantly inhibit P-gp mediated transport of CPT-11 and SN-38 in MDCKII cells (27). However, thalidomide does not seem to interact directly with P-gp, neither as a substrate nor as an inhibitor (28). Thus a regulation of *ABCB1* gene expression by thalidomide could be hypothesised as it has been shown that a clinical treatment with this drug induced *ABCB1* extinction in a patient with refractory myeloma (9).

DNA methylation represents an important epigenetic mechanism of expression control with an inverse relationship between promoter CpG methylation and *ABCB1* expression (18,19,29). The methylation status of the *ABCB1* gene promoter sites was thus analysed. It appears that, at the levels of the -50 and -100 GC boxes, *ABCB1* promoter display an unmethylated profile in 8226 drug-sensitive cells. This promoter hypomethylation in 8226 cells suggest that *ABCB1* repression in drug-sensitive cells could be independent of its promoter methylation, a phenomenon previously reported in H69 and SW620 cells (10,17). On the contrary, 8226-Dox40 drug-resistant cell promoter GC boxes were fully methylated. We reported previously a similar *ABCB1* promoter hypermethylation in human ovarian drug-resistant OV1-VCR cell lines (11). This phenomenon was also observed in K562/ADM resistant cells and treatment of these cells with the demethylating agent 5-azacytidine resulted in *ABCB1* down-regulation (30). These data therefore suggest an important role for the -110GC box whose methylation in 8226-Dox40 cells could result in the impairment of the repressor binding site. Conversely, an inverse correlation between *ABCB1* gene expression and methylation has been observed in AML (31). However, the relationships between *ABCB1* methylation and expression levels appear rather complex as some tumours display different characteristics and, for instance, *ABCB1* methylation has been shown to increase with tumour aggressiveness in prostate carcinomas (32,33). Moreover, correlations have been found between *ABCB1* promoter methylation status and genetic polymorphisms C3435T and G2677T/A (34).

Further treatments with thalidomide or PGA did not result in any change in these methylation profiles in 8226 cells. The data observed in the present study also indicate that short-term treatments of 8226 cells with 250 μM thalidomide or PGA do not induce any change in *ABCB1* gene expression. Recent analyses on DNA microarrays indicate that *ABCB1*, unlike *ABCB2*, does not seem to appear among the genes whose expression could be significantly modified *in vivo* in myelomas by a short exposure to thalidomide or lenalidomide (35). Conversely, our data show that these treatments induce significant down-regulation of *VEGF* gene expression as already reported in other cell types (36). 8226 cells are known to express numerous growth factors, including HGF, PDGF and VEGF, but not bFGF (37). Our data indicate that 8226-Dox40 cells exhibit the same expression of *VEGF* than drug-sensitive 8226 cells. Moreover, thalidomide and PGA induced similar *VEGF* expression decrease in both cell lines, indicating that thalidomide could down-regulate *VEGF* expression in drug-resistant myeloma cells as already reported for cisplatin-resistant human lung carcinoma cells (38).

In conclusion, in 8226-Dox40 drug-resistant myeloma cells, a short-term treatment with thalidomide alters chromatin supra-organisation. These textural changes induced by both thalidomide and PGA appear independent from the effects on gene expressions tested so far: *ABCB1* whose expression remains unaffected in drug-resistant cells as well as for VEGF, since the expression of this gene decreased both in drug-sensitive and -resistant cells, although Thalidomide induced chromatin alterations in 8226-Dox40 cells only. Thus, these data emphasize the complex relationships between chromatin texture regulation and gene expression in tumour cells.

Acknowledgements

This work was supported by grants from the Comités départementaux de la Haute-Marne et de la Marne de la Ligue Française contre le Cancer. We wish to thank Professor W.S. Dalton for 8226 and 8226-Dox40 cell lines.

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