Differences in Nuclear Positioning of 1q12 Pericentric Heterochromatin in Normal and Tumor B Lymphocytes with 1q Rearrangements

Leila Barki-Celli,[1†] Christine Lefebvre,[1†] Patricia Le Baccon,[1‡] Gwenael Nadeau,[1] Thierry Bonnefoix,[1] Yves Usson,[2] Claire Vourc'h,[3] Saadi Khochbin,[3] Dominique Leroux,[1] and Mary Callanan[1*]

[1]Lymphoma Research Group, INSERM E353, Institut Albert Bonniot—Faculté de Médecine, Université Joseph Fourier Grenoble I, Domaine de la Merci, La Tronche Cedex, France
[2]CNRS, UMR5525, Institut d'Ingénierie et de l'Information de Santé (IN3S), Université Joseph Fourier Grenoble I, Domaine de la Merci, 38706 La Tronche Cedex, France
[3]INSERM U309, Institut Albert Bonniot - Faculté de Médecine, Université Joseph Fourier Grenoble I, Domaine de la Merci, 38706 La Tronche Cedex, France

The frequent rearrangement of chromosome band 1q12 constitutive heterochromatin in hematologic malignancies suggests that this rearrangement plays an important pathogenetic role in these diseases. The oncogenic mechanisms linked to 1q12 heterochromatin are unknown. Constitutive heterochromatin can epigenetically regulate gene function through the formation of transcriptional-silencing compartments. Thus, as a first step toward understanding whether 1q12 rearrangements might compromise such activity in tumor cells, we investigated the 3-D organization of the 1q12 heterochromatin domain (1q12HcD) in normal and tumor B lymphocytes. Strikingly, in normal B cells, we showed that the 1q12HcD dynamically organizes to the nuclear periphery in response to B-cell receptor engagement. Specifically, we observed an almost twofold increase in 1q12Hc domains at the extreme nuclear periphery in activated versus resting B lymphocytes. Remarkably, 1q12Hc organization was noticeably altered in tumor cells that showed structural alterations of 1q12; the 1q12Hc domains were significantly displaced from the extreme nuclear periphery compared to normal activated B lymphocytes ($P > 0.0001$), although overall peripheral localization was maintained. In a case in which there was a translocation of *IGL* enhancer to 1q, the altered nuclear positioning of the 1q12HcD was even more pronounced (5% of the 1q12Hc domains at the nuclear periphery compared to 20% in other lymphoma lines), and we were able to mimic this effect in two additional B-cell tumor lines by treatment with trichostatin A, a histone deacetylase (HDAC) inhibitor. Taken together, these results point to the 1q12HcD having a specific, nonrandom, and regulated peripheral organization in B lymphocytes. This organization is significantly disrupted in lymphoma cells harboring 1q rearrangements.

INTRODUCTION

Cancer cells harbor chromosomal abnormalities that can alter gene expression and function. In particular, B-cell malignancies have recurrent chromosomal translocations that have been shown by molecular characterization to deregulate the expression and function of specific genes that control lymphoid homeostasis (Willis and Dyer, 2000). A common mechanism that is a part of this gene activation process is the juxtaposition of enhancer elements derived from immunoglobulin loci with the promoters of target genes. An important, though not as yet very precisely characterized, facet of enhancer activity is the ability to open up chromatin to regulatory factors. As such, immunoglobulin gene enhancer–linked chromosomal translocations have been intensively studied and have come to represent a paradigm of abnormal gene activation in cancer cells.

Recently, we and others have described a novel category of chromosomal aberrations that involves constitutive heterochromatin on human chromosome 1 (cytogenetic band 1q12; Busson-Le Coniat et al., 1999; Le Baccon et al., 2001; Itoyama et al., 2002). These rearrangements are observed at high frequency in B-cell non-Hodgkins lymphoma, B-cell acute leukemias, and multiple myeloma. There also is evidence of the involvement of 1q12 heterochromatin in certain solid tumors (Mertens et al., 1997). In this setting, it is likely that these

Supported by: INSERM; Association pour la Recherche Contre le Cancer (ARC); Région Rhone-Alpes: Thématiques prioritaires "Cancer"; Fondation de France.

†Contributed equally to this work.

*Correspondence to: Mary Callanan, Lymphoma Research Group, INSERM E353, Institut Albert Bonniot, Université Joseph Fourier Grenoble 1, Domaine de la Merci, 38706 La Tronche Cedex, France. E-mail: mary.callanan@ujf-grenoble.fr

‡Present address: CNRS, UMR 218, Institut Curie-Recherche, Pavillon Pasteur, 26 rue d'Ulm, 75248 Paris Cedex 05.

Received 13 August 2004; Accepted 5 January 2005

DOI 10.1002/gcc.20179

Published online 21 April 2005 in Wiley InterScience (www.interscience.wiley.com).

rearrangements play an important role in the onset or progression of these diseases.

These chromosomal rearrangements have been shown to have a number of striking features. First, they involve gain of 1q sequences including 1q12 heterochromatin and the formation of 1q12 heterochromatin/euchromatin junctions. We hypothesized that these junctions might result in repression of gene expression through position effects at the chromosomal breakpoints. It is also possible that excess 1q12 heterochromatin in tumor nuclei results in the formation of aberrant transcriptionally silent domains that might sequester transcriptional regulators or chromatin remodeling factors in the affected cells (Le Baccon et al., 2001). It has been shown that 1q12 heterochromatin can recruit transcriptional regulators such as Ikaros and members of the Polycomb family (Saurin et al., 1998; Sewalt et al., 2002). Interaction with Ikaros has been shown to require the DNA binding domain, whereas Polycomb recruitment requires functional SUVAR39H1 histone methyltransferase activity (Saurin et al., 1998; Sewalt et al., 2002).

Evidence exists that, at least in yeast, perinuclear localization of heterochromatin facilitates heterochromatin-induced transcriptional silencing (Andrulis et al., 1998). It is also clear that genes are nonrandomly positioned in the nucleus and that proximity to heterochromatin is correlated with transcriptional activity (Cremer and Cremer, 2001; Chubb and Bickmore, 2003; Spector, 2003). For instance, it has been shown that the gene-rich human chromosome 19 preferentially localizes to the nuclear interior, whereas the relatively gene-poor chromosome 18 is localized to the nuclear periphery (Croft et al., 1999), although this organization appears to be partially lost in tumor cells (Cremer et al., 2003). Differential nuclear positioning of genes down- or up-regulated during B- and T-lymphocyte development has been described. These include the *RAG1* and *RAG2* genes during B- and T-lymphocyte development and cytokine genes during TH1 or TH2 polarization in T-lymphocyte differentiation (Brown et al., 1997, 1999; Grogan et al., 2001). Specific positioning of the immunoglobulin genes *IGH* and *IGK* during murine B-cell development also has been shown (Kosak et al., 2002). Finally, the organization of chromosome territories appears to be largely conserved in higher primates (Tanabe et al., 2002) and to depend on both cell type (Alcobia et al., 2000, 2003; Cremer et al., 2003) and cell-cycle stage (Vourc'h et al., 1993; Walter et al., 2003).

In this context, we investigated the spatial organization of 1q12 heterochromatin within the lymphocyte nucleus. We chose to study 1q12 heterochromatin organization in both ex vivo cultured human peripheral-blood B lymphocytes and B-cell tumor lines with and without 1q12 heterochromatin rearrangements. We also investigated the impact of histone deacetylase inhibitor trichostatin A on this organization.

MATERIALS AND METHODS

Cells and Cell Lines

Peripheral-blood B lymphocytes were obtained from healthy donors by using CD19-positive selection (CD19 Dynabeads, Dynal Biotech, Compiègne, France) and separation on a Percoll gradient to obtain activated and resting primary B lymphocytes. Resting lymphocytes either were used directly for immunofluorescence confocal microscopy or were activated in vitro using an anti-IgM antibody (Jackson ImmunoResearch, West Grove, PA) at 5 μg/ml for 48 hr in RPMI complete medium in order to stimulate the B-cell receptor (BCR).

The cell lines studied were follicular lymphoma cell lines B593, CH1, and RL and Burkitt lymphoma cell lines Wien 133 and BL136. The B593 line was established in the laboratory as previously described (Callanan et al., 2000); the RL cell line was obtained from the ATCC; the Wien 133 cell line was as described previously (Zani et al., 1996) and was a gift from Dr. Martin Dyer (Department of Haematology, University of Leicester, Leicester, United Kingdom); and the BL136 cell line was obtained from IARC (Lyon, France). The karyotypes of the cell lines are given in Table 1.

The CH1 cell line will be described in detail in another article (Barki-Celli, in preparation). Briefly, the CH1 cell line was obtained by xenotransplantation in SCID mice of tumor cells derived from the lymph node biopsy of a 41-year-old patient (CJL) diagnosed with grade III follicular lymphoma. Tumors were passaged in vivo for 4 months, at which time the animals were killed and the tumor cells harvested. Immunophenotypic, cytologic, cytogenetic, and molecular analyses (*IG* gene rearrangements) confirmed identity with the original patient tumor material. The CH1 cell line was established in vitro from these tumor cells. Immunophenotypic, cytogenetic, and *IGL* results have remained stable during repeated passaging in vitro in the laboratory.

All cell lines except CH1, which was cultured in 10% human AB+ serum, were cultured in RPMI supplemented with 10% or 15% (BL136 cells) fetal calf serum in a 5% saturated CO_2 atmosphere.

TABLE 1. Karyotypes of the B-Cell Tumor Lines Used for Confocal Microscopy Analysis

Cell line[a]	Tumor type[b]	Karyotype[c]
RL	FL	47,X,-Y,add(3)(q21),add(9)(q34),add(13)(q34),add(13)(q34),**t(14;18)(q32;q21)**,−18,−19,−20,+21,+4 mar / 94,idem × 2
B593	FL	47,X,-Y,**t(1;22)(q22;q11)**,+7,**t(8;14;18)(q24;q32;q21)**,t(9;15)(?p21;?q23),del(16)(q21q24)[14]
CH1[d]	DLBCL	44,XY,del(1)(p34),der(1)t(1;22)(q21;q11),**der(2)t(1;2)(q12;p22)**,der(3),der(4)t(2;4)(p23;q3?5),der(5)t(5;11)(q35;?),t(6;11)(q1?6;p1?3),der(8;12)(q10;q10),**t(14;18)(q32;q21)**der(14;21)(q10;q10),ins(16;16)(q?;q?q?),−22
BL136	BL	46,XY,**dup(1)(q11q41)**;add(4)(q35);**t(8;14)(q24;q32)**,add(15)(q26)
Wien 133	BL	47,XY,**der(6)t(1;6)(q12;q?12)**,+7,add(8)(p12),**t(8;14;12)(q24;q32;q24)**[16]

[a]Sources of cell lines are given in the Materials and Methods section.
[b]FL: follicular lymphoma; DLBCL: diffuse large B-cell lymphoma; BL: Burkitt lymphoma.
[c]Both the chromosomal abnormalities that involve 1q12 heterochromatin and those that correspond to known primary rearrangements are indicated in bold in each cell line. In the B593 cell line, the t(1;22) involving the FCGR2B and IGL genes is also in bold.
[d]Revised karyotype after M-FISH analysis.

Preparation of Nuclei for Immuno-FISH and Confocal Microscopy

For confocal microscopy experiments, the cells were diluted to 0.5 million per milliliter in RPMI medium 16 hr prior to the harvesting and preparation of 3-D preserved cell nuclei for FISH. Nuclei were prepared essentially as described previously (Ferguson and Ward, 1992). Briefly, lymphocytes were harvested and resuspended at a concentration of 2.5×10^6/2 ml of nuclei buffer (5 mM HEPES, 50 mM KCL, 10 mM $MgSO_4$, 0.05% Tween 20, pH 8) with 30 µl of RNase (10 mg/ml). Lymphocytes were then permeabilized by the addition of 50 µl of Triton X-100 (10% v/v) for 10 min at 4°C, incubated for 30 min at 37°C, and fixed in paraformaldehyde (PFA 5%, 1× PBS) under agitation. The reaction was stopped by the addition of 30 ml of Tris-HCl, 100 mM (pH 7.0). The released nuclei were collected by centrifugation on a Ficoll gradient and washed 3 times in 1× PBS. Nuclei (50,000) were then cytocentrifuged onto vectabond-treated glass slides. These slides were stored for a maximum of 3 weeks prior to use in the immuno-FISH experiments.

Trichostatin A Treatment

Trichostatin A (Sigma-Aldrich, Saint Quentin, Fallavier, France) (TSA) was used at a concentration of 50 ng/ml for the RL cell line and 75 ng/ml for the BL136 cell line for 16 h. These concentrations were identified as effective for histone deacetylase (HDAC) inhibition as evaluated by increased histone H4 acetylation and as nontoxic on the basis of dose–response tests in both cell lines. Toxicity was assessed by monitoring of cell viability using both cell counts and flow cytometry following Annexin V/propidium iodide double staining. Histone H4 acetylation levels after trichostatin A treatment were assessed by immunofluorescence on fixed cells and by extraction of nuclear proteins and by Western blotting using an acetyl-H4 antibody (ChIP grade, rabbit polyclonal anti-H4 acetylated at lysines 5, 8, 12, and 16, Upstate Biotechnology, Waltham, MA).

For immunofluorescence, cells were resuspended at 0.1×10^6 in 100 µl/1× PBS, then cytocentrifuged onto glass slides. Nonspecific sites were blocked in 1% nonfat milk/1× PBS, then incubated with anti-acetyl-H4 antibody diluted 1/150 in 1% nonfat milk, 1× PBS. Anti-acetyl-H4 was detected using a TRITC-conjugated secondary antibody (diluted 1/50 in 1% nonfat milk, 1× PBS).

For Western blotting, total nuclear protein lysates were prepared. Briefly, 5×10^6 cells were washed twice in ice-cold 1× PBS. Cell pellets were resuspended directly in 1–2 times the volume of 1× loading buffer (2% SDS/1% β-mercaptoethanol/0.06 M Tris, pH 6.8/10% glycerol/1% bromophenol blue) and sonicated at 120 watts for 1 min. Samples were then boiled for 5 min prior to loading onto 15% SDS-PAGE gels. Gel loading was checked by Coomassie staining.

Chromatin organization following TSA treatment was assessed by staining with 4′,6-diamidino-2-phenylindole (DAPI). Dense DAPI-staining chromatin corresponds to heterochromatin.

Immuno-FISH

The 1q12 heterochromatin region was detected using the pUC1.77 probe. The nuclear membrane was detected by a goat polyclonal anti–lamin B antibody (Santa Cruz, Biotechnology, Waltham,

MA). Prior to immuno-FISH, slides were rinsed twice in 2× SSC, then permeabilized (Triton X-100/Saponine 0.5%/PBS 1×) twice for 10 min to allow penetration of the probe. Nonspecific sites were blocked in 3% BSA/2× SSC for 30 min at 37°C. Slides were then rinsed in 2× SSC and incubated for 10 min in 50% formamide/2× SSC. For FISH, nuclei were preincubated with biotin- or digoxigenin-labeled probe in hybridization buffer for 30 min prior to codenaturation of the probe and target for 5 min at 86°C. For simultaneous detection of DNA and protein targets, in blocking buffer (3% BSA/4SSC/0.1% Tween 20), appropriate dilutions were utilized of streptavidin-Alexa 488 (1/300 dilution, Vector Laboratories, Burlingame, CA) for biotin-16-dUTP-labeled probes, rhodamine-coupled anti-digoxigenin antibody (Roche Diagnostics, Meylan, France, 1/200 dilution) for digoxigenin-11-dUTP-labeled probes, and a goat anti–lamin B polyclonal, followed by cyanine V-labeled rabbit antigoat antibody (Jackson ImmunoResearch, 1/100 and 1/150 dilutions, respectively) for lamin-B detection.

Confocal Microscopy Image Acquisition and Data Analysis

Confocal images were obtained on a Zeiss LSM 410 equipped with helium/neon (emission at 543 and 633 nm) and argon lasers (emission at 488 and 514 nm) using an NA 1.4/63× objective. Image acquisition was performed using LSMPC software. For each nucleus, 80 optical sections were captured at 0.2-μm intervals. For each sample, optical sections were obtained from 50 nuclei, except for the experiments with TSA, for which 30 nuclei were examined. Confocal images were processed and analyzed with ana3D and edit3D custom software as described previously (Parazza et al., 1993). After image segmentation, nuclear and signal volumes and radial distances from the geometric nuclear center were calculated. Radial distances from the nuclear edge were normalized to avoid biases from variation in nuclear size. The nuclear periphery thus had a value of 0 and the nuclear center a value of 1. Maintenance of nuclear morphology after FISH was evaluated using a method described previously, that is, nuclear sphericity was determined on the basis of measurements of nuclear volume and surface area (Parazza et al., 1993).

Data Validation and Statistical Methods

To determine whether the radial distance distribution of 1q12 domains was random, experimental distributions were compared to random distributions for the same objects using Monte Carlo simulations. Both experimental and Monte Carlo simulation data were binned using a linear radial scale (classes of increasing volume). The bin boundaries were chosen such that the width of each bin was not less than the smallest visible spot (diffraction-limited spot) obtainable by a light microscope (approximately 0.2 μm) but large enough to smooth out nonsignificant random variations. Comparison of the distributions of 1q12 domains between cell lines was then performed using the Kolmogorov–Smirnov (KS) test. This is a nonparametric test that allows comparison of continuous distributions. The threshold for significant differences was set to 0.02 in all experiments except for those involving treatment with TSA ($P = 0.05$).

RESULTS

1q12 Organization in Resting and Activated Normal Human B Lymphocytes

Heterochromatin has been shown to organize into discrete nuclear regions, notably the nuclear periphery, where it is thought to function in the formation of transcriptional silencing compartments (Spector, 2003). In this setting, we investigated the organization of 1q12 pericentric heterochromatin in normal human peripheral-blood B lymphocytes and in B-cell tumor lines with and without 1q12 rearrangements.

Strikingly, normal resting B lymphocytes showed a marked peripheral nuclear localization of the 1q12HcD, which became even more pronounced following BCR engagement: the proportion of 1q12 signals rose to 54% in the extreme nuclear periphery (0–0.1 distance interval) in activated B cells compared to 29% in resting B lymphocytes (KS, $P = 0.005$; compare Fig. 1B and D).

Although nuclear volume was significantly increased in activated B lymphocytes compared to resting B lymphocytes (276.8 $\mu m^3 \pm 28.4$ in resting B lymphocytes vs. 318.6 $\mu m^3 \pm 36.4$ in activated B cells; KS, $P < 0.001$), no detectable increase in 1q12HcD volume was observed (8.8 μm^3 in resting B lymphocytes compared to 9.2 μm^3 in activated B lymphocytes). Likewise, association or clustering of 1q12Hc domains was infrequent in either case.

The experimentally determined distribution of 1q12 heterochromatin domains differed significantly from that predicted by a Monte Carlo simulation of random 1q12 heterochromatin distribution in a spherical nucleus. Monte Carlo simulations

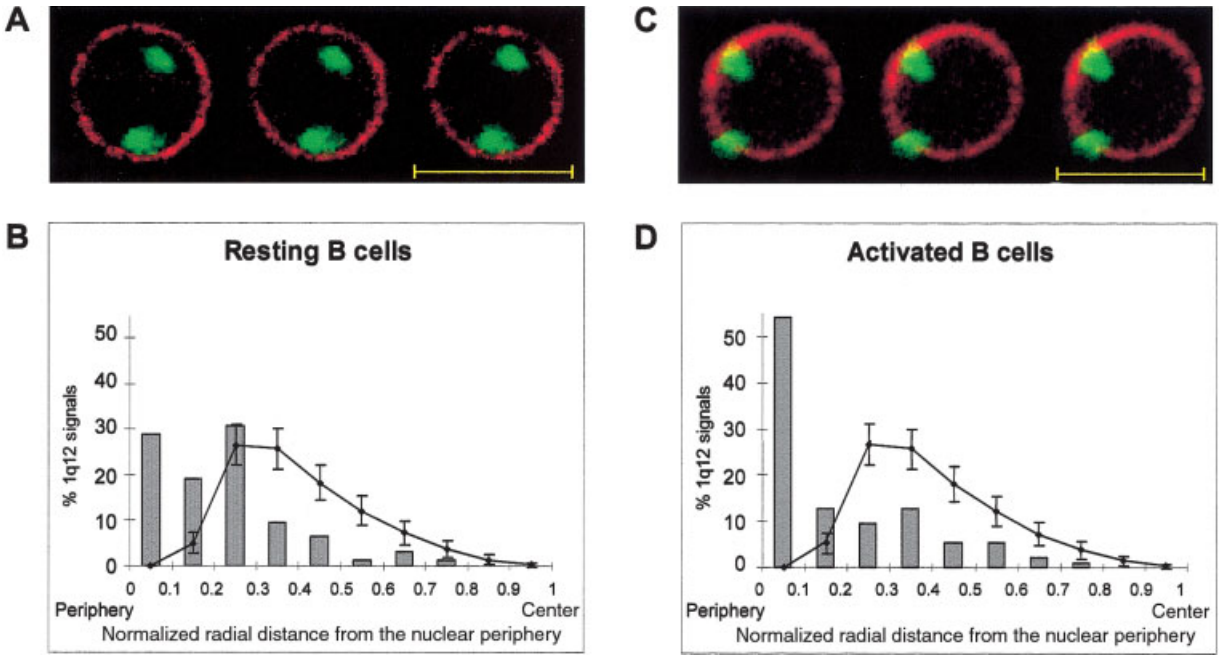

Figure 1. 1q12 heterochromatin domains dynamically localized to the nuclear periphery in normal human peripheral-blood B lymphocytes. (**A**) Serial optical sections from the nuclei of normal resting human B lymphocytes (0.2 μm intervals). (**B**) Radial nuclear distributions of 1q12 heterochromatin domains in normal resting B lymphocytes (gray histograms). (**C**) Serial optical sections from the nuclei of normal resting activated human B lymphocytes (0.2 μm intervals). The nuclear membrane was detected by an anti–lamin B antibody (red signal) and 1q12 domains were detected by a pUC1.77 probe (green signal). Scale bars, 10 μm. (**D**) Radial nuclear distributions of 1q12 heterochromatin domains in normal activated B lymphocytes (gray histograms). Radial distances were normalized such that 0 represents the nuclear periphery and 1 represents the center of the nucleus. The black line represents a Monte Carlo simulation of a random radial distribution of 1q12 domains.

predicted a peak 1q12 heterochromatin domain distribution to be in the 0.2–0.3 nuclear interval (25% of domains), whereas experimental distributions showed a peak in the 0.0–0.1 and 0.1–0.2 nuclear intervals. Thus, it appears that the radial distribution of the 1q12 heterochromatin domains in the B lymphocytes was nonrandom (Fig. 1B and D).

Human B-Lymphoid Tumor Lines with 1q12 Rearrangements Show Altered Spatial Organization of 1q12 Heterochromatin

Chromosome band 1q12 rearrangements result in quantitative and qualitative changes in 1q12 heterochromatin, that is, increased 1q copy number and 1q12 heterochromatin/euchromatin breaks. This might be expected to alter 1q12 domain organization and function within the nucleus. To determine whether this occurred in the cells studied, we analyzed 1q12 heterochromatin organization in the B-cell tumor lines that showed 1q12 heterochromatin rearrangements.

We chose to examine cell lines that showed unbalanced translocations to either chromosome 2 or 6 in the CH1 or Wien 133 cell lines, respectively, or 1q12 duplication in the BL136 cell line (see Table 1 for karyotypes). These cell lines thus showed 3 copies of the 1q12 heterochromatin region (Le Baccon et al., 2001). Unbalanced translocations involving 1q12 heterochromatin represent the most common type of structural change involving this region in diffuse large B-cell and follicular lymphomas, whereas 1q12 segmental duplications are particularly frequent (up to 40% of cases in some published series) in Burkitt lymphoma (Le Baccon et al., 2001).

In the present study, the radial distribution of 1q12 heterochromatin in human B-cell tumor lines with 1q12 rearrangements was altered significantly compared to that in normal B lymphocytes (KS, $P < 0.0001$; Fig. 2 A–C). Specifically, activated B lymphocytes were found consistently to have a higher proportion of 1q12 domains in the 0–0.1 distance interval (54%) than were the tumor cell lines with 1q12 rearrangements (approximately 20%; Fig. 2 A–C). However, if nuclear intervals of 0–0.2 were considered, overall localization of 1q12 domains to the nuclear periphery was maintained in tumor cells with 1q12 rearrangements, that is, more than 40% of 1q12 signals were localized to these intervals.

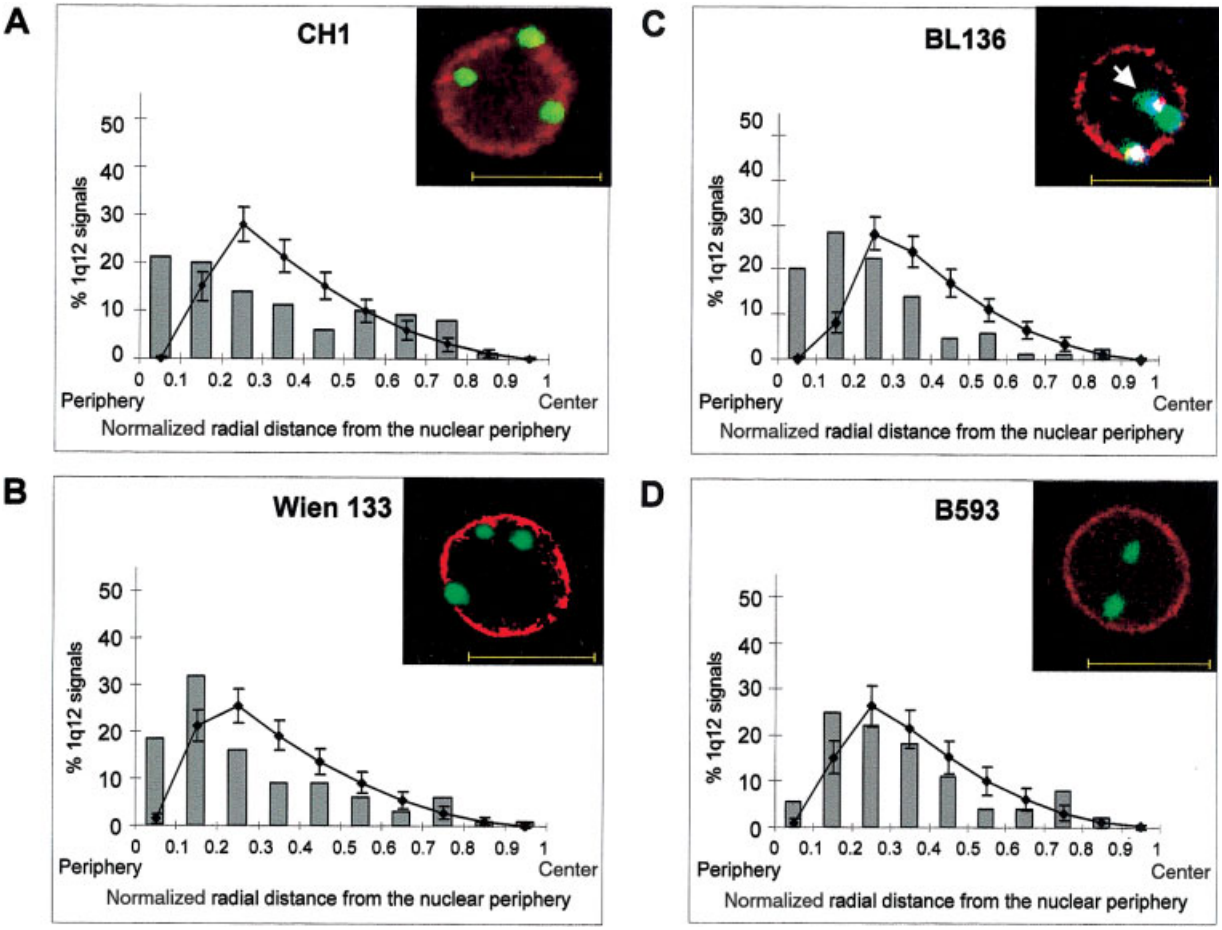

Figure 2. 3-D organization of 1q12 heterochromatin domains in B-cell tumor lines with 1q12 heterochromatin rearrangements. Shown are radial nuclear distributions of 1q12 domains (gray histograms) for cell lines (**A**) CH1, (**B**) Wien 133, (**C**) BL136, and (**D**) B593. Representative single-equatorial optical sections are shown for each cell line (insets). The nuclear membrane was detected by an anti–lamin B antibody (red signal), 1q12 domains were detected by a pUC1.77 probe (green signal), and chromosome 1 centromeres (panel C only) were detected by a CEP1 probe (Vysis; blue signal). Monte Carlo simulations of random radial 1q12 distributions for each cell line are plotted in black. Note the presence of two peripheral 1q12 heterochromatin domains of differing volumes in the BL136 cells. The larger domain corresponds to the duplicated chromosome 1 because one large pUC1.77 signal colocalized with a single chromosome 1 centromere signal (white arrow, inset of C).

Despite structural rearrangement of 1q12 heterochromatin in the tumor cells, cases of association between 1q12Hc domains on different chromosomes were rare (less than 2% of nuclei). However, it is worth remarking that in BL136 cells, the duplicated 1q12 domains usually appeared as a single large-volume hybridization signal that was tightly associated with a single chromosome 1 centromere signal [>19% of signals from the dup(1q) chromosome were observed as a single large signal]. This clustering of 1q12Hc domains was somewhat unexpected because physical mapping has shown the germ-line and duplicated 1q12 regions to be separated by a distance of more than 70 Mb on this duplicated chromosome 1 (Le Baccon et al., 2001).

IGL Enhancer Translocation to Chromosome Arm 1q and HDAC Inhibition by TSA Are Associated with Aberrant Spatial Organization of 1q12 Heterochromatin in Lymphoma Cells

Physical linkage of gene sequences to enhancer elements can modify their spatial positioning within the nucleus (Francastel et al., 1999, 2001; Tumbar and Belmont, 2001). In this context, we analyzed the 1q12HcD radial distribution in a follicular lymphoma–derived cell line that showed juxtapositioning of the *IGL* enhancer (from 22q11) to the *FCGR2B* gene in 1q22 (approximately 18 Mb distal from 1q12, according to the chromosome 1 sequencing map). This occurred as a consequence of a balanced t(1;22) translocation that we previously have shown to result in

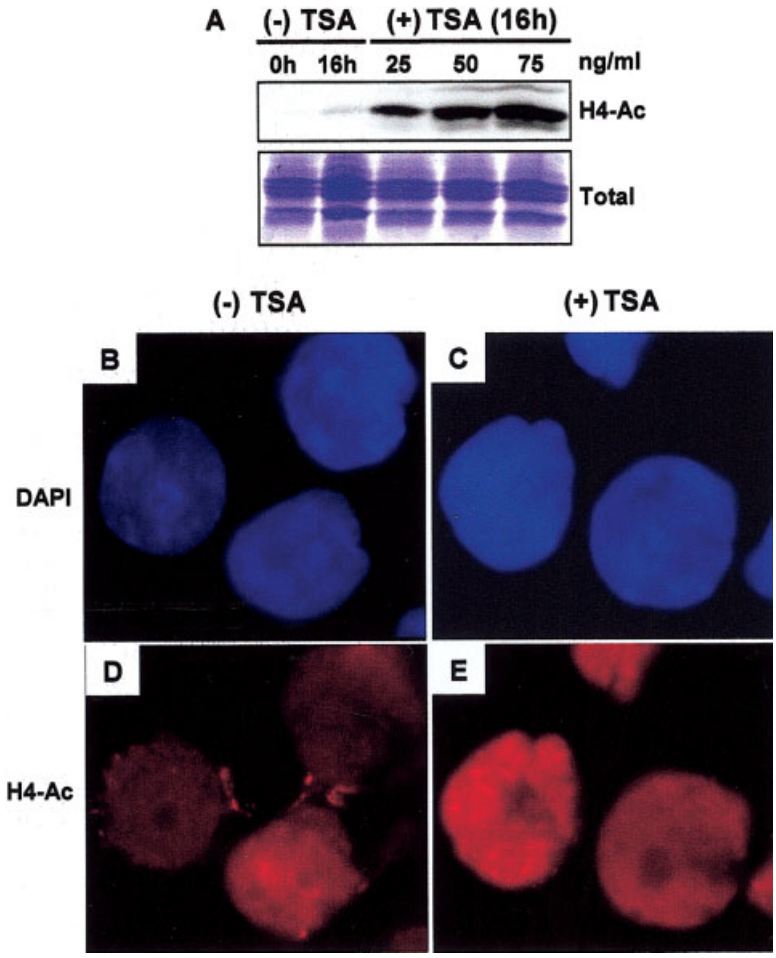

Figure 3. Treatment with a histone deacetylase inhibitor (TSA) induced hyperacetylation of histone H4 in BL136 cells. (**A**) Western blot analysis of H4 lysine acetylation in nuclear lysates from BL136 cells treated with TSA for 16 h (Coomassie staining presented as a control for gel loading); (**B**) DAPI staining of chromatin in untreated BL136 cells; (**C**) DAPI staining of chromatin in TSA-treated BL136 cells; (**D**) immunofluorescence detection of histone H4 acetylation in the nuclei of untreated BL136 cells; (**E**) immunofluorescence detection of histone H4 acetylation in the nuclei of TSA-treated BL136 cells.

high-level constitutive expression of *FCGR2B* (Callanan et al., 2000).

Remarkably, pronounced differences were observed in the 1q12 distribution patterns of the B593 cells compared to those of the other tumor cell lines and of the normal human B lymphocytes: significantly fewer 1q12 domains (only 5%) were localized to the 0–0.1 nuclear interval in the B593 cells than in the other B-cell tumor lines (on average 20% of 1q12 signals were localized to the 0–0.1 nuclear interval) or in the normal B cells (KS test; B593 versus Wien 133 and BL136 cell lines: $P < 0.0001$ and $P = 0.017$, respectively; B593 versus activated B lymphocytes: $P < 0.0001$). This was taken to reflect displacement of the translocated chromosome 1 copy. Interestingly, the apparent differences between the B593 and CH1 cells in 1q12HcD distribution were not found to be statistically significant. In this context, it is worth noting that the CH1 cell line also showed other chromosome 1 rearrangements not involving 1q12 (Table 1).

To determine whether the apparent effects of *IGL* enhancer activity could be mimicked by agents that favor increased histone acetylation, we investigated the effects of the HDAC inhibitor TSA on 1q12 organization in the BL136 cell line and a second follicular lymphoma cell line (RL) that does not present chromosome 1 rearrangement. As expected, treatment of the RL and BL136 cell lines with TSA at 50 or 75 ng/ml, respectively, for 16 hr resulted in increased acetylation of H4 (data not shown and Fig. 3, respectively). Subsequently, confocal microscopy analysis in the TSA-treated RL and BL136 cells revealed significant disruption of the organization of 1q12 heterochromatin domains. Specifically, fewer 1q12 heterochromatin signals were observed in the extreme nuclear periphery compared to those observed in the untreated cells (Figs. 4 and 5, panels B and C). In the RL cells, the proportion of 1q12HcD in the 0–0.1 nuclear interval (50%) was reduced to 28% after TSA treatment. It should be noted that the RL 1q12HcD radial distribution closely resembles that observed in normal activated B lymphocytes. Likewise, in untreated BL136 cells, 25% of 1q12 domains were localized

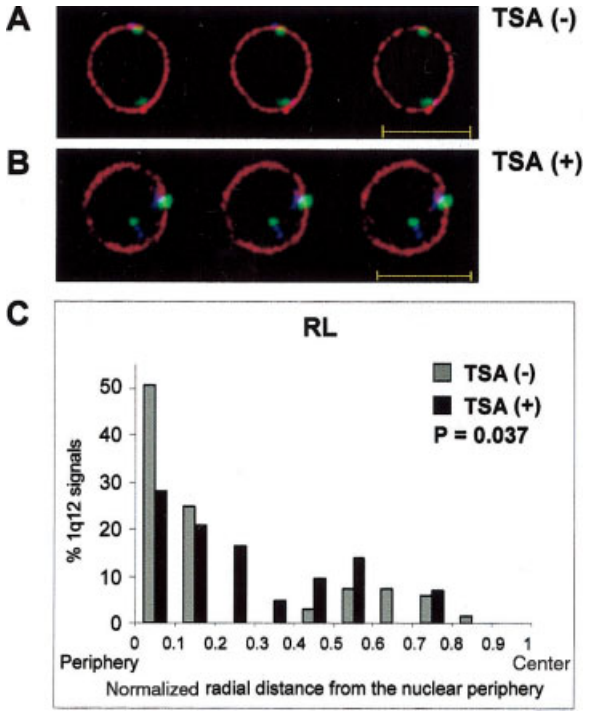

Figure 4. HDAC inhibitor treatment perturbs 1q12 heterochromatin domain organization in RL cells. Serial optical sections through the nuclei of (**A**) untreated and (**B**) TSA-treated RL cells. The nuclear membrane was detected by anti–lamin B antibody (red), and 1q12 heterochromatin domains were detected using the pUC1.77 probe (green signals). Scale bars, 10 μm. (**C**) Radial distributions of 1q12 domains before (gray bars) and after (black bars) TSA treatment. For each condition, data were obtained from 30 nuclei (80 optical sections obtained per nuclei).

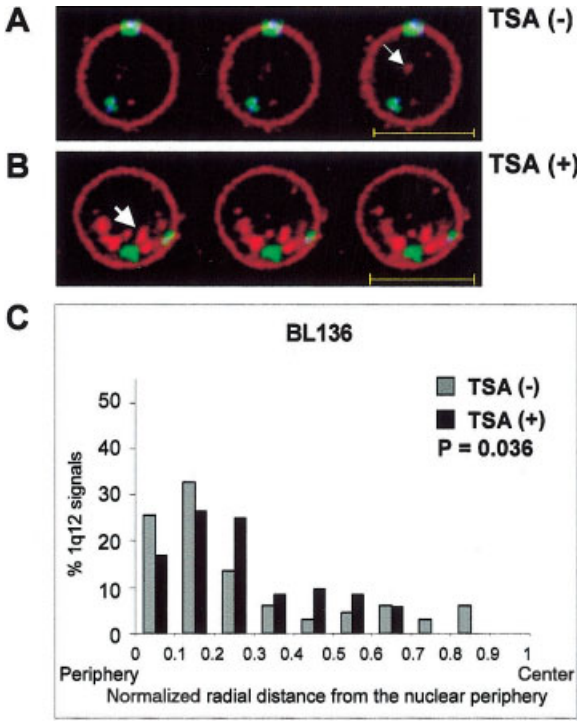

Figure 5. HDAC inhibitor treatment perturbs 1q12 heterochromatin domain organization in BL136 cells. Serial optical sections through the nuclei of (**A**) untreated and (**B**) TSA-treated BL136 cells. Experimental details were as indicated in the Figure 4 caption. Note the presence of lamin B foci in the nuclei of BL136 cells (A and B, white arrows). These lamin B foci were more pronounced in TSA-treated BL136 cells (B). (**C**) Radial distributions of 1q12 domains before (gray bars) and after (black bars) TSA treatment. For each condition, data were obtained from 30 nuclei (80 optical sections obtained per nuclei).

to the 0–0.1 interval, compared to 12% in the same interval in the TSA-treated cells. Taken together, these data suggest that nuclear events that favor increased histone acetylation either locally (enhancer activity) or globally (HDAC inhibition) contribute to the regulation of 1q12 heterochromatin organization in B lymphocytes.

TSA treatment in either cell line was not associated with increased 1q12 signal volume or aberrant association of 1q12 heterochromatin domains in these cells (not shown). However, in the TSA-treated BL136 cells (but not in the RL cells) lamin B nuclear distribution was markedly altered: lamin B immunostaining was observed in the form of foci that were displaced from the nuclear membrane (Fig. 5B). Faint lamin B foci also were visible in some untreated BL136 cells but not in RL cells (Figs. 4A and 5A).

DISCUSSION

As a first step toward understanding the potential epigenetic impact of qualitative and quantitative alterations affecting the satellite II-rich 1q12 heterochromatin region in lymphoma cells, we examined its nuclear organization in both normal and tumor human B lymphocytes. To our knowledge, this is the first detailed characterization of the nuclear organization of a specific pericentric heterochromatin region in human cells.

It was shown that 1q12 heterochromatin has a strong bias toward peripheral nuclear localization in human B lymphocytes. This organization is nonrandom, does not involve clustering of individual 1q12 heterochromatin domains, and appears to be regulated throughout the cell cycle: B-cell activation, through antigen receptor engagement, resulted in marked relocalization of 1q12 domains to the extreme nuclear periphery. Remarkably, this organization was significantly perturbed in tumor cell lines that showed 1q12 heterochromatin rearrangements to other chromosome sites, although overall localization to the nuclear periphery was maintained. Finally, we showed that nuclear events that favor increased local or global chromatin acetylation (enhancer activity or HDAC inhibition) were associated with abnormal spatial positioning of 1q12 heterochromatin.

Relocalization of constitutive heterochromatin to the nuclear periphery after B-cell activation has not been described previously and constitutes rather unique behavior. Lymphocyte activation is associated with global chromatin acetylation and can be visualized as up to a 5- to 10-fold increase in nuclear volume (Zhao et al., 1998). In contrast, increased localization of 1q12 constitutive heterochromatin toward the nuclear periphery would be expected to require targeted histone deacetylation and methylation (Rea et al., 2000; Nishioka et al., 2002) that would favor associations with the inner nuclear membrane (HP1/lamin B-receptor associations, for example; Kourmouli et al., 2000; Makatsori et al., 2004). Indeed, in this study, we showed that HDAC inhibition was associated with displacement of 1q12 constitutive heterochromatin domains away from the nuclear periphery.

Regulated 1q12 relocalization to the nuclear periphery after B-cell activation thus would be expected to involve specific pathways that reinforce the epigenetic information required for 1q12 heterochromatin interactions with the inner nuclear membrane while allowing global, BCR-dependent chromatin remodeling to proceed. A good candidate for initiating such a process would be Ikaros, which plays a key role in lymphopoiesis (Busslinger, 2004). It is known to be selectively recruited to pericentric heterochromatin—together with chromatin remodeling activities and HDACs—during lymphocyte activation (Brown et al., 1997, 1999; Kim et al., 1999) and has been shown to be specifically enriched in 1q12 heterochromatin in interphase nuclei (Saurin et al., 1998).

We have demonstrated the existence of regulated peripheral localization of 1q12 heterochromatin domains in normal B lymphocytes, and we have shown that this organization is partially lost in tumor cells that harbor 1q12 rearrangements. This is of particular interest in view of studies that show perinuclear localization of chromatin as a means of facilitating transcriptional silencing, at least in yeast (Andrulis et al., 1998). It has also become evident that dynamic heterochromatin/gene associations occur during epigenetic regulation of gene activity in mammalian cells (Brown et al., 1997, 1999; Grogan et al., 2001; Chubb and Bickmore, 2003; Su et al., 2004).

Interestingly, during thymocyte maturation, *TDT* gene silencing involves early repositioning to pericentric heterochromatin, and this precedes establishment of a silent chromatin state at the *TDT* promoter (Su et al., 2004). Furthermore, relocalization to pericentric heterochromatin was not observed in a T-cell line model of thymocyte differentiation, and *TDT* repression in these cells was reversible (Su et al., 2004). This might indicate a functional requirement for relocalization to pericentric heterochromatin for *TDT* gene silencing (stable repression) in vivo. Our findings suggest that at least some of the positional information required for pericentric heterochromatin-associated gene silencing may be lost in tumor cells.

We have shown that B cells in which 1q12 heterochromatin is linked in *cis*, albeit at a distance, to an immunoglobulin gene enhancer show a markedly different distribution of 1q12 heterochromatin compared to other tumor cells or indeed to normal B lymphocytes: 1q12 heterochromatin was displaced toward the nuclear interior. This suggests that enhancer activity and, by extension, gene activity/chromatin acetylation in the proximity of constitutive heterochromatin regions can dominantly modify heterochromatin positioning in the nucleus. In keeping with this, 1q12 heterochromatin displacement from the nuclear periphery also was observed after TSA treatment. The relocalization of 1q12HcD in cells with *IGL* enhancer translocations was reminiscent of data showing enhancer-dependent repositioning of genes/transcriptional regulators to the nuclear interior or to euchromatic regions enriched in transcriptional transactivating factors (Francastel et al., 1999, 2001; Tumbar and Belmont, 2001).

In the present study, the peripheral nuclear organization of 1q12 heterochromatin was significantly disrupted after TSA treatment. As mentioned earlier, the simplest explanation for this effect is that HDAC inhibition directly interfered with establishment of the epigenetic codes required for localizing 1q12 heterochromatin to the nuclear membrane. However, effects on the acetylation of other nuclear factors required for peripheral nuclear localization of heterochromatin cannot be excluded. Indeed, pronounced redistribution of lamin B as intranuclear foci was observed in the BL136 cells after TSA treatment. Although intranuclear lamin A and/or B staining has been observed in the G1 phase of human (Maske et al., 2003), mouse (Moir et al., 2000), and CHO (Broers et al., 1999) cells, its functional significance is not currently known. Posttranslation modifications such as farnesylation, endoproteolysis, and methylation at a carboxy-terminal CAAX motif have been reported to control lamin B interactions with the inner nuclear membrane (Maske et al., 2003). Whether HDACs also are involved has not been investigated.

The observed displacement of 1q12 heterochromatin domains away from the nuclear periphery in

tumor cells with 1q rearrangements (of 1q12 or more distal sites) may reflect the influence of partner chromosomes involved in these rearrangements. As an example, a previous study that investigated the positioning of the normal and translocated copies of chromosomes 18 and 19 involved in a t(18;19) showed alterations of the nuclear position of the translocated chromosomal segments, although such segments tended to maintain their orientation toward their most common localization (Croft et al., 1999). In the chromosome 18 segment, this was toward the nuclear periphery, the preferred localization of normal chromosome 18), whereas the chromosome 19 segment was positioned toward the nuclear interior, the preferred position of normal chromosome 19 (Croft et al., 1999). Interestingly, two-color FISH analysis using 1q12 and chromosome 2 centromere probes in the CH1 cell line indicated preferred localization of the normal chromosome 2 centromere toward the nuclear interior (Barki-Celli, manuscript in preparation). This may explain why 1q12Hc domains tend to be displaced toward the nuclear interior in these cells. The same may be true for other 1q12 rearrangements, depending on the partner chromosome involved.

Apart from one exception—duplication of 1q12 heterochromatin on the same chromosome 1 [dup(1q)]—association of 1q12 heterochromatin domains was only rarely observed in either normal or tumor nuclei in this study. In the case of 1q12 duplication studied here, previous work showed that the duplicated 1q12 segments were separated by at least 70 Mb (duplication breakpoint localized to 1q41; Le Baccon et al., 2001). In the interphase nucleus, these duplicated 1q12 domains thus could have been expected to appear as two unassociated domains. Instead, a majority of nuclei showed duplicated 1q12 signals that appeared as a single large domain. This tends to suggest that 1q12 domains can associate in *cis* over very long distances, possibly through a chromatin "looping" mechanism. Indeed, gene activation at the murine β-globin locus has been shown to involve contact between active genes and the β-globin LCR by a chromatin looping-out mechanism covering more than 60 kb (Tolhuis et al., 2002). Likewise, immunoglobulin gene loci that are in the process of active V(D)J recombination undergo locus contraction through looping of individual immunoglobulin gene subdomains (Roldan et al., 2005).

The 1q12 rearrangements have been shown to involve more than 20 partner chromosomes, none of which are immunoglobulin gene loci (Le Baccon et al., 2001). Recent data concerning the dynamics of DNA radiation-induced double-strand breaks (DSBs) in human cells offer an explanation (Aten et al., 2004). Briefly, it has been shown that DSB-containing chromosome domains are mobile and can form clusters to which the DSB repair proteins MREII and RAD51 are recruited, and errors in the repair process within these clusters have been proposed to favor erroneous recombination events between DSB-containing chromosomes in a dynamic "breakage firs" model for chromosomal rearrangements in tumor cells (Aten et al., 2004). Frequent recruitment of breakage-prone repetitive regions of the genome, such as 1q12, to DSB repair clusters might lead to an increased risk of errors in DSB repair and thereby to chromosomal rearrangements.

In summary, we have identified a specific nonrandom, regulated peripheral nuclear organization for 1q12 constitutive heterochromatin in both normal and tumor B lymphocytes. This organization may support an as-yet-unidentified function for 1q12 heterochromatin in the epigenetic control of normal and leukemic B-lymphocyte development and function.

ACKNOWLEDGMENTS

Leila Barki-Celli has been the recipient of doctoral stipends from both the French Ministry for Research and Technology and the Ligue Nationale Contre le Cancer. We are particularly grateful to Dr. Karine Monier for her invaluable technical advice on 3D FISH.

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