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Frequent hypermethylation of *DBC1* in malignant lymphoproliferative neoplasms

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Allelic loss at chromosome 9q31–34 is a frequent event in many lymphoproliferative malignancies. Here, we examined *DBC1* at 9q33.1 as a potential target in lymphomagenesis. DBC1 is a putative tumor suppressor that has been shown to be involved in the regulation of cell growth and programmed cell death. The methylation status of the *DBC1* promoter CpG island was examined by methylation-specific PCR, bisulfite sequencing, and methylation-specific melting curve analysis. *DBC1* was hypermethylated in 5 of 5 B-cell-derived lymphoma cell lines, 41 of 42 diffuse large B-cell lymphomas, 24 of 24 follicular lymphomas, 5 of 5 mantle cell lymphomas, 4 of 4 small lymphocytic lymphomas, 1 of 2 lymphoplasmacytoid lymphomas, and in 12 of 12 acute lymphoblastic leukemias, but was unmethylated in 1 case of splenic marginal zone lymphoma, in 12 of 12 multiple myelomas, in 24 of 24 reactive lymph nodes, and in 12 of 12 samples of blood lymphocytes from random donors. *DBC1* hypermethylation was associated with transcriptional silencing in lymphoma cell lines, and reexpression of this gene could be induced by treatment with the demethylating agent, 5-aza-2′-deoxycytidine. Our data suggest that hypermethylation of the *DBC1* promoter region is a frequent event during the development of lymphoproliferative malignancies, and that *DBC1* hypermethylation may serve as a marker for these cancers.
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Loss of heterozygosity (LOH) at the long arm of chromosome 9 has been demonstrated in several types of lymphoid malignancies, including diffuse large B-cell lymphoma,[1–3] follicular lymphoma,[4] multiple myeloma,[5] mantle cell lymphoma,[6] Hodgkin lymphoma,[7] acute lymphoblastic leukemia,[8,9] and chronic lymphocytic leukemia.[10] Some of these studies have implicated the 9q32–33 region as the prevailing target for deletion, suggesting that this region harbors an important tumor suppressor gene for lymphoproliferative cancers. The same region is lost in several other types of human cancers[11] with particularly high frequencies reported for transitional cell carcinomas of the bladder.[12]

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Fine mapping analysis in bladder cancer led to the identification of a putative tumor suppressor gene at 9q33.1, which has been designated as *DBC1* (deleted in bladder cancer 1).[13] The 5′ region of *DBC1* contains a CpG island that is aberrantly hypermethylated in approximately 50% of bladder cancer cell lines and tumors,[13,14] 40% of oral squamous cell carcinomas,[15] 80% of non-small cell lung cancer cell lines, and a proportion of primary non-small cell lung cancer tumors.[16] In bladder cancer, low-density *DBC1* promoter methylation was shown to occur in an age-related manner in the matched normal tissue, indicating that *DBC1* promoter methylation may constitute an early event in carcinogenesis.[13,14]

In humans, DBC1 is expressed at high levels in the brain and spinal cord[13] as well as in mesenchymal stem cells.[17] In a previous study, we have shown that *DBC1* is silenced by promoter methylation in spontaneously transformed hTERT-transduced mesenchymal stem cells (TERT-20 cells), lending further support to a tumor-suppressive role

of *DBC1*.[17] Notably, *DBC1* hypermethylation occurred after loss of the *INK4A/ARF* locus in these cells, and silencing of *DBC1* was associated with the ability to form tumors in mice. However, the DBC1-regulated molecular pathway(s) that exerts a tumor-suppressive effect in stem cells, and possibly other cell types, has not yet been fully elucidated. The protein structure of DBC1 shows some resemblance to perforin (the membrane-attack complex; amino acids 72–251), suggesting that DBC1 may be involved in pore formation during cell lysis. Interestingly, perforin has been shown to be involved in both familial hemophagocytic lymphohistiocytosis[18] and sporadic lymphoma by point mutations.[19] Ectopic reexpression of DBC1 in cancer cell lines with *DBC1* hypermethylation elicited a range of cellular effects, including cell-cycle arrest, caspase-independent apoptosis,[20] and altered expression of key factors in the plasminogen pathway.[21] However, it is still not evident which of these DBC1 functions are essential in controlling carcinogenesis, and under which cellular circumstances they are active.

Here, we have examined *DBC1* as a candidate tumor suppressor in lymphoid tumors. We show that the *DBC1* 5′ CpG-island is unmethylated in normal blood lymphocytes and reactive lymph nodes, but hypermethylated in 91 of 92 cases of diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, small lymphocytic lymphoma, acute lymphoblastic leukemia and lymphoma cell lines, and in 1 of 2 cases of lymphoplasmacytoid lymphoma, suggesting that inactivation of *DBC1* is a frequent event in lymphomagenesis. Transcription from a hypermethylated *DBC1* promoter in lymphoma cell lines could be induced by treating the cells with the demethylating agent, 5-aza-2′-deoxycytidine.

Materials and methods

Specimens

Lymphoma biopsies were obtained from 42 cases of diffuse large B-cell lymphoma and 24 cases of follicular lymphoma, including 12 *de novo* and 12 relapsed cases, 5 cases of mantle cell lymphoma, 4 cases of small lymphocytic lymphoma, 2 cases of lymphoplasmacytoid lymphomas and 1 case of splenic marginal zone lymphoma. Bone marrow specimens were sampled from 12 cases of multiple myeloma, and 12 cases of acute lymphoblastic leukemia, including 7 pre-B-acute lymphoblastic leukemias and 5 T-acute lymphoblastic leukemias. Reactive lymph nodes were obtained from 24 individuals, and peripheral blood lymphocytes were obtained from 12 random, anonymous donors. RNA from normal human brain was purchased from Ambion. Approval of this study was obtained from the local ethical committees.

Cell Culture and 5-aza-2′-Deoxycytidine Treatment

Diffuse large B-cell-derived lymphoma cell lines, Farage, Pfeiffer, DB1, HT, and RL, were purchased from the American Type Culture Collection (ATCC), and maintained in RPMI 1640 medium with Glutamax supplemented with 10% fetal calf serum. Cells were treated for 72 h with 5-aza-2′-deoxycytidine at a final concentration of 0.5 μM.

Reverse Transcriptase Polymerase Chain Reaction

RNA from cell lines, reactive lymph nodes and primary lymphoma samples was extracted using the NucleoSpin Kit (Macherey-Nagel). One microgram of RNA was reverse transcribed using Superscript III (Invitrogen) and subsequently amplified by PCR using previously described primers.[13] Quantitative PCR was performed using the LightCycler 1.0 instrument (Roche) in 10-μl volumes containing 1 μl of cDNA, 5 pmol of each primer, 3 mM $MgCl_2$ and 1 × FastStart DNA Master SYBR Green I Kit (Roche). PCR conditions were 10 min at 95°C to activate the enzyme followed by 35 cycles of denaturation at 95°C for 5 s, primer annealing at 65°C for 10 s and primer elongation at 72°C for 15 s. Primers for DBC1 amplification were as described.[13] GAPDH primers, AGGGGGGAGCCAAAAGGG (sense) and GAGGAGTGGGTGTCGCTGTTG (antisense) were used for normalization. RNA from normal brain and early passage TERT-20 cells was used as positive control; RNA from late passage TERT-20 cells was used as negative control.

Allelic Loss of *DBC1*

Genomic DNA was extracted according to standard procedures. Allelic loss at the *DBC1* locus was identified by analysis of the c.618C/T biallelic polymorphism in exon 5 of *DBC1*, using a combination of PCR and denaturing gradient gel electrophoresis (DGGE).[22] The primers were: 5′-[CGCCCGCCGC GCCCCGCGCCCGTCCCGCCGCCCCCGCCCG]-TCTC ACACCTATCTTATTTGATATCTAC-3′ and 5′-[CCCG CC]-ACTTTTTCCACTGGCTTCCT-3′. PCR was performed in 15-μl reactions containing 10 mm Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM cresol red, 12% sucrose, 10 pmol of each primer, 100 μM each dNTP, 100 ng of DNA, and 0.8 units of HotstarTaq polymerase. The cycling parameters were: 35 cycles at 95°C (20 s), 55°C (20 s), and 72°C (30 s). PCR products were analyzed in a 10% denaturant/6% polyacrylamide-70% denaturant/12% polyacrylamide double-gradient gel (100% denaturant = 7 M urea and 40% formamide). The gel was run at 80 V for 16 h in 1 × TAE buffer kept at a constant temperature of 56°C. In informative cases, a semi-quantitative estimation of allelic loss was done by evaluating the intensities of the two homoduplex bands. After ethidium bromide

staining and UV transillumination, a TIFF-format image of the gel was generated and analyzed using the 1D Gel Analysis Phoretix Software (Phoretix, Newcastle, UK).

Promoter Hypermethylation of *DBC1*

The methylation status of the *DBC1* promoter was examined using three different approaches: methylation-specific PCR (MS-PCR),[23] methylation-specific melting curve analysis (MS-MCA)[24] and bisulfite genomic sequencing.[25] For all three methods, DNA was treated with sodium bisulfite, which converts unmethylated cytosine to uracil but leaves methylated cytosines unchanged.[26] MS-PCR was performed using previously described primers and conditions.[15]

For MS-MCA, two primer sets were used. The first set (5′-GTGGGAATTTGGGAGAGTTTT-3′ and 5′-AA TATAAACCAAACTACTAAAAACCAAATA-3′) amplifies a 100-bp region (including 7 CpG sites) of the *DBC1* 5′CpG island (nt. +41 to nt. +140 from the initiation ATG site), and the other set (5′-TAAA TAGTGTTAAATATTTATAGAGAGA-3′ and 5′-CCC AAATCCTAATACCCTTAAA-3′) amplifies a 182-bp region (including 13 CpG sites) from nt. −710 to nt. −528 (GenBank Accession no. NT_008470).

Amplification was carried out using the LightCycler 1.0 instrument (Roche) in 10-μl volumes containing 1 μl of the bisulfite-treated DNA, 5 pmol of each primer, 3 mM $MgCl_2$ and 1 × FastStart DNA Master SYBR Green I Kit (Roche). PCR conditions were 10 min at 95°C to activate the enzyme followed by 35 cycles of denaturation at 95°C for 5 s, primer annealing at 62°C for 10 s and primer elongation at 72°C for 15 s. Subsequently, melting curves were obtained by measuring the drop in fluorescence when raising the temperature from 70 to 98°C at 0.05°C/s. The melting peaks were calculated using the LightCycler software 4.05 as described.[24]

For bisulfite genomic sequencing, nucleotides −771 to −528 of *DBC1* were amplified from bisulfite-reacted DNA using the primers 5′-AGAGA AGTTTTTGTTTTATTTTG-3′ and 5′-CCCAAATCCT AATACCCTTAAA-3′. Direct sequence analysis of PCR products was performed with a ^{33}P-end-labeled internal primer 5′-TGTTTTATTTTGGGAGGTT-3′ using the ThermoPrime Cycle Sequencing Kit (Amersham Life Science, Cleveland, OH, USA), according to the manufacturer's instructions.

Results

Allelic Loss of *DBC1* in Diffuse Large B-Cell Lymphoma

To investigate whether deletions of the 9q33 region in lymphoid neoplasms include *DBC1*, we examined a single-base polymorphism in exon 5 of this gene. Fourteen of 30 diffuse large B-cell lymphomas were heterozygous for this marker, which allowed us to evaluate allelic losses in these tumors by resolving the two alleles by DGGE and measuring the band intensities. Four of the tumors (29%) showed unequal allelic distribution, suggesting that one of the *DBC1* alleles had been lost in the tumor cells (Figure 1).

DBC1 Promoter Hypermethylation in Lymphoproliferative Malignancies

To examine the methylation status of *DBC1* in lymphoid malignancies, we first examined 12 diffuse large B-cell lymphomas using MS-PCR. Bisulfite-treated DNA was used as template in two separate reactions with primers that specifically amplify either methylated or unmethylated *DBC1*. Positive signals for both methylated and unmethylated *DBC1* were obtained for all 12 samples (See Figure 2 for examples).

To exclude potential methodological artifacts, the same samples were examined using MS-MCA. First, a region of the *DBC1* 5′ CpG-island containing 7 CpG sites was amplified from bisulfite-treated DNA using primers that do not discriminate between methylated and unmethylated molecules, and the PCR products were subsequently subjected to melting analysis. In concordance with the results of the

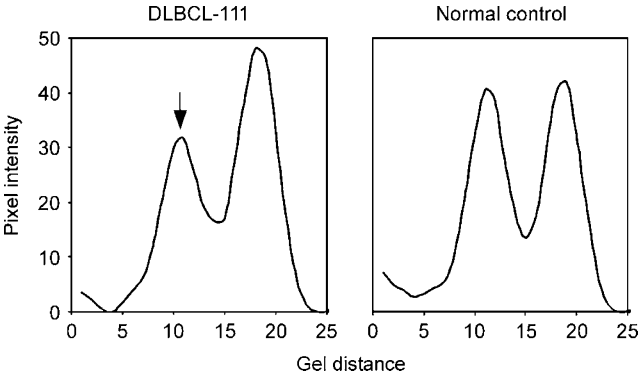

Figure 1 DGGE-based detection of *DBC1* allelic loss. Diffuse large B-cell lymphoma (DLBCL) no. 111 shows loss of one of the alleles of the c.618C/T polymorphism. Pixel intensity and gel distance are in arbitrary units.

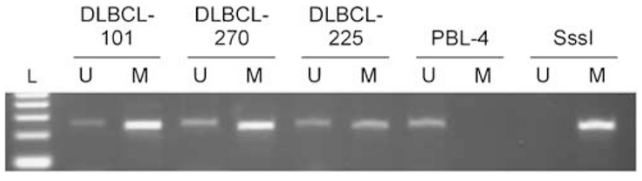

Figure 2 MS-PCR analysis of the *DBC1* promoter in diffuse large B-cell lymphoma. DNA was treated with sodium bisulfite and PCR-amplified with primer pairs specific for unmethylated (lanes U) and methylated (lanes M) *DBC1* alleles. *Sss*I-methylated DNA provided a positive and peripheral blood lymphocytes (PBL) a negative control for methylated *DBC1*.

MS-PCR analysis, all diffuse large B-cell lymphomas showed a peak with an apparent melting temperature (T_m) of approximately 84°C, corresponding to the peak obtained with *Sss*I-methylated DNA, whereas peripheral blood lymphocytes showed a single melting peak with a T_m~4°C lower (Figure 3a). Most diffuse large B-cell lymphomas also showed the lower-melting peak, probably originating from non-malignant cells in these samples.

Next we used the MS-PCR and MS-MCA assays to examine the methylation status of *DBC1* in a broader spectrum of lymphoid neoplasms, including 30 additional diffuse large B-cell lymphomas, 12 primary follicular lymphomas, 12 relapsed follicular lymphomas, 5 mantle cell lymphomas, 4 small lymphocytic lymphomas, 2 lymphoplasmacytoid lymphomas, 1 splenic marginal zone lymphoma, 12 acute lymphoblastic leukemias, 12 multiple myelomas, and 5 cell lines (Farage, Pfeiffer, DB1, HT, and RL) that were established from patients with diffuse large B-cell lymphoma. In total, *DBC1* hypermethylation was detected in 41 of the diffuse large B-cell lymphomas (98%), 24 of the follicular lymphomas (100%), 5 of the mantle cell lymphomas (100%), 12 of the acute lymphoblastic leukemias (100%), 4 of the small lymphocytic lymphomas (100%), 1 of the lymphoplasmacytoid lymphomas (50%) and 5 of the cell lines (100%) (Table 1). Three of the small lymphocytic lymphomas and the methylated lymphoplasmacytoid lymphoma showed a single melting peak with a melting temperature between the peaks for fully methylated and unmethylated DNA, suggesting that the promoter was heterogeneously methylated in these cases. The cell lines contained only hypermethylated *DBC1* alleles (data not shown), suggesting that the methylation is either biallelic or monoallelic in combination with loss of the other *DBC1* allele. All 12 multiple myelomas and the case of splenic marginal zone lymphoma contained only unmethylated *DBC1* (Table 1).

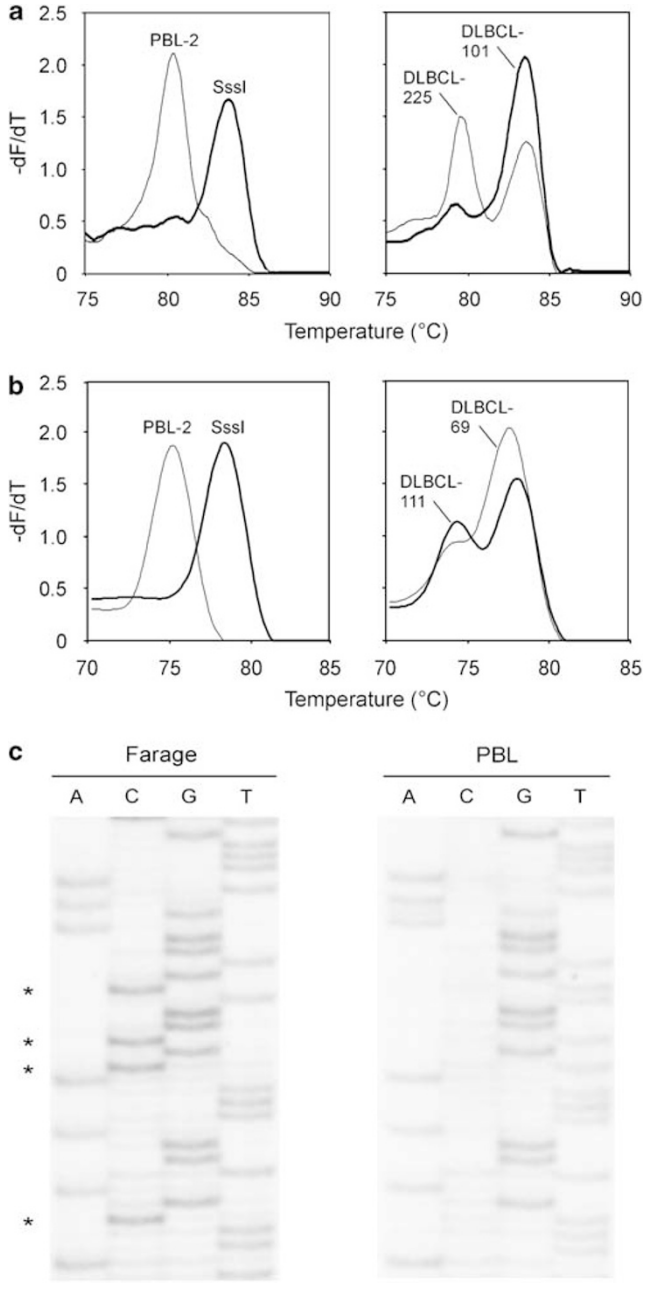

Figure 3 Methylation analysis of the *DBC1* promoter in diffuse large B-cell lymphoma and lymphoma cell lines. Examples of MS-MCA are shown for the proximal (**a**) and distal (**b**) part of the promoter. (**c**) Bisulfite genomic sequencing of the distal part of the *DBC1* promoter. CpG sites are indicated by asterisks.

Recently, the activity of the *DBC1* promoter was suggested to be associated with the methylation status of a region upstream of the sequence examined in our first experiments.[16] We, therefore, also examined the methylation status of this region by MS-MCA and bisufite genomic sequencing. All five lymphoma cell lines and six of seven diffuse large B-cell lymphomas, which all showed a fully methylated proximal promoter region, were also fully methylated in the distal part of the promoter (Figure 3b). The remaining diffuse large B-cell lymphoma showed heterogeneous methylation of the distal part of the *DBC1* promoter. Similar to the analysis of the proximal part of the promoter, most of the primary diffuse large B-cell lymphomas also showed a low-melting peak corresponding to unmethylated *DBC1*. Bisulfite genomic sequencing of the distal part of the promoter in the five lymphoma cell lines and in normal and fully methylated controls confirmed the MS-MCA data (Figure 3c).

To examine the possible correlation between promoter hypermethylation and transcriptional silencing, as has been demonstrated for a great number of tumor suppressor genes,[27] we analyzed RNA from 12 primary diffuse large B-cell lymphomas using real-time quantitative RT-PCR. As positive controls, we used RNA from human brain and TERT-20 cells.[28] These cells express DBC1 at early passages, but lack expression at later passages.[17] Using this PCR assay, which easily detected RNA from TERT-20 cells diluted 1000-fold, we were

Table 1 Methylation status of *DBC1* in malignant lymphoid neoplasms, reactive lymph nodes, and blood lymphocytes from random donors

Specimen	*Number of cases*	*Age median (range)*	*Number of cases with DBC1 hypermethylation*
Lymphoma cell lines	5	NA	5 (100%)
Diffuse large B-cell lymphoma	42	65 (26–94)	41 (98%)
Follicular lymphoma	24	49 (33–80)	24 (100%)
Acute lymphoblastic leukemia	12	22 (7–63)	12 (100%)
Mantle cell lymphoma	5	66 (59–86)	5 (100%)
Small lymphocytic lymphoma	4	58 (47–84)	4 (100%)
Lymphoplasmacytoid lymphoma	2	50/81	1 (50%)
Multiple myeloma	12	58 (41–77)	0 (0%)
Reactive lymph nodes	12	28 (18–41)	0 (0%)
	12	70 (56–78)	0 (0%)
Peripheral blood lymphocytes	12	NA	0 (0%)

NA, not available.

unable to detect DBC1 transcripts in any of the primary lymphoma samples (data not shown).

DBC1 is Unmethylated in Normal Peripheral Blood Lymphocytes and Reactive Lymph Nodes

To examine whether *DBC1* promoter hypermethylation was specific for malignant lymphoid cells, we first examined peripheral blood lymphocytes from 12 random donors. Both the MS-PCR and MS-MCA assays generated only signals for unmethylated *DBC1*. We also examined DNA isolated from 24 reactive lymph nodes, which were removed because of suspected malignancy. These specimens included uninvolved lymph nodes from patients with malignant melanoma, and reactive lymph nodes from patients with infectious diseases or chronic inflammation. Eight of these lymph nodes showed a remarkable follicular hyperplasia that, by histology, could be misinterpreted as follicular lymphoma. None of the 24 reactive lymph nodes showed any evidence of aberrant *DBC1* methylation, as determined by MS-PCR and MS-MCA (Table 1).

We also examined the expression of DBC1 in seven reactive lymph nodes and in six peripheral blood lymphocyte samples from normal donors. None of these peripheral blood lymphocytes or lymph nodes showed expression of DBC1 mRNA by RT-PCR or real-time quantitative PCR analysis (data not shown). Thus, considering that the *DBC1* promoter is unmethylated in peripheral blood lymphocytes (Figures 2 and 3) and reactive lymph nodes, DBC1 expression may be blocked in normal lymphocytic cells by a methylation-independent mechanism.

Reexpression of DBC1 in Lymphoma Cell Lines

To examine whether promoter methylation of *DBC1* is required to suppress transcription in lymphoma cells, we treated five cell lines with the demethylating agent, 5′-aza-2-deoxycytidine. None of the untreated cell lines expressed DBC1 mRNA, but expression was activated in all cell lines after treatment with 5′-aza-2-deoxycytidine (Figure 4). These data indicate that demethylation of the *DBC1* promoter may be sufficient to induce DBC1 expression in lymphoma cells and, accordingly, that the factor(s) suppressing DBC1 expression in normal lymphocytes is lost in the malignant counterparts.

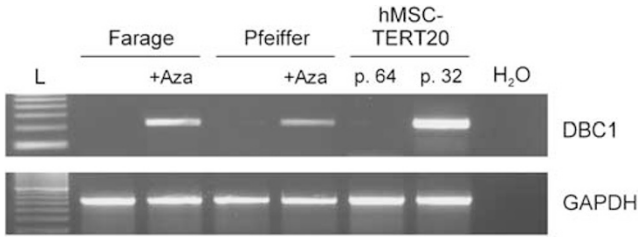

Figure 4 DBC1 expression in lymphoma cell lines. RT-PCR analysis of a 232-bp DBC1 transcript (exons 7–8) in lymphoma cells lines with and without treatment with 5-aza-2′-deoxycytidine (Aza; 0.5 μM) for 3 days. Early and late passage TERT-20 cells were used as positive and negative controls, respectively. Amplification of GAPDH transcripts is shown as a control for RNA quality. L, 100-bp ladder.

DBC1 Hypermethylation in Lymphoid Malignancies is Age Independent

Previous studies have shown that promoter CpG islands of some tumor suppressor genes can become hypermethylated in an age-dependent manner.[29] Specifically, hypermethylation of the *DBC1* promoter has been demonstrated in normal urothelium from men >50 years old.[14] Since the incidence of lymphoma is increasing with age and is highest among individuals aged >60 years,[30] we considered whether a similar age-related mechanism might explain the high frequency of *DBC1* hypermethylation in lymphoproliferative malignancies. However, the lymphoma and acute lymphoblastic leukemia patients harboring *DBC1* hypermethylation in their tumors showed a wide age distribution, with >10%

of the lymphoma patients and >80% of the acute lymphoblastic leukemia patients aged <40 years. Furthermore, *DBC1* hypermethylation was not detected in multiple myeloma patients aged up to 77 years or in reactive lymph nodes from a group of 12 elderly (median 70 years; range 56–78). These data suggest that *DBC1* hypermethylation in lymphoma is cancer specific and occurs independent of age.

Discussion

Here, we have shown that the *DBC1* promoter is hypermethylated in lymphoma cell lines and in virtually all cases of diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, small lymphocytic lymphomas, acute lymphoblastic leukemia, and in a fraction of lymphoplasmacytoid lymphomas, but not in multiple myeloma specimens, reactive lymph nodes, or peripheral blood lymphocytes from healthy individuals. In addition, allelic loss of *DBC1* was found in roughly one-third of diffuse large B-cell lymphomas. Collectively, these data suggest that inactivation of *DBC1* by promoter hypermethylation and/or deletion is an almost obligate event in diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, small lymphocytic lymphoma, and acute lymphoblastic leukemia. Using MS-PCR, San José-Enériz *et al*[31] recently demonstrated *DBC1* hypermethylation in 29/141 acute lymphoblastic leukemias and 4/4 acute lymphoblastic leukemia cell lines.

A direct role of *DBC1* as a tumor suppressor has been questioned because its expression is suppressed in most normal human cells, with brain tissue,[13] lung,[16] and early passage mesenchymal stem cells[17] as notable exceptions. Recent transfection studies with full length DBC1 in a bladder cancer cell line showed inhibition of population growth, but no increases in cell death.[21] However, other attempts to generate cells with stable DBC1 expression have repeatedly failed, indicating that expression of DBC1 may lead to severe growth inhibition or even cell death, at least in some cellular contexts. Indeed, Wright *et al*[20] showed that induced expression of DBC1 in bladder tumor cell lines induced caspase-independent cell death. Accordingly, activation of DBC1 expression may be important for the elimination of some normal cells, possibly acting as a fail-safe mechanism that is triggered in parallel with other death pathways or when other death pathways fail.

Several other tumor suppressor genes have been shown to be hypermethylated in lymphoma, although the prevalence of hypermethylation for individual tumor suppressor genes rarely exceeds 60%.[32] However, Reddy *et al*[33] recently showed that the *SHP1* promoter region is almost universally methylated in lymphomas and leukemias. Methylation frequencies at or close to 100% have also been reported for a set of genes involved in B-cell signaling in Hodgkin disease.[34]

According to the current paradigm, hypermethylation of tumor suppressor genes is acquired earlier or later in the tumorigenic process. Nevertheless, considering that hypermethylation of the *DBC1* promoter occurred in almost 100% of diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, small lymphocytic lymphoma, and acute lymphoblastic leukemia, we cannot exclude that *DBC1* hypermethylation may be an inherent epigenetic feature of the cells from which these tumors are derived. The cellular origin of the individual lymphoid malignancies is still unknown, although the present lymphoma classification is based on the assignment of tumors to their presumed normal counterparts in the normal lymphocyte differentiation pathways. Other theories suggest that cancers may originate from a common population of epigenetically marked stem or progenitor cells. In early cell differentiation, when methylation fluctuates, non-neoplastic stem or progenitor cells may achieve specific growth or survival advantages by the particular epigenetic signature.[35,36] This expanded population of epigenetically altered, yet polyclonal progenitor cells may thus have obtained an oncogenic potential that makes them more vulnerable to secondary tumorigenic events.

Whether *DBC1* promoter hypermethylation is acquired during lymphomagenesis or is a normal epigenetic characteristic of certain hematologic precursor cells, it may serve as a marker to discriminate between benign lymphoid hyperplasia and malignant lymphoproliferation. While a negative *DBC1* methylation test does not exclude malignancy and will require further analysis, a lymph node that is positive for *DBC1* methylation is likely to represent malignancy. If indeed *DBC1* is a tumor suppressor that is important in the control of lymphopoiesis, *DBC1* may be a potential target for lymphoma treatment since transcriptional silencing by DNA methylation can be reversible controlled by inhibitors of methylation.[37]

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Disclosure/conflicts of interest

The authors have no conflicts of interest to declare.

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