PRDM1 is a tumor suppressor gene in natural killer cell malignancies

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Edited* by Janet D. Rowley, University of Chicago Medical Center, Chicago, IL, and approved November 2, 2011 (received for review September 17, 2011)

Natural killer cell lymphoma (NKCL) constitutes a rare and aggressive form of non-Hodgkin lymphoma, and there is little insight into its pathogenesis. Here we show that *PRDM1* is a tumor suppressor gene in NKCLs that is inactivated by a combination of monoallelic deletion and promoter CpG island hypermethylation. We observed monoallelic deletion of *PRDM1* loci in 8 of 18 (44%) NKCL cases. The other allele showed significant promoter methylation in 12 of 17 (71%) cases. In support of its role as a tumor suppressor gene, the reconstitution of PRDM1 in PRDM1-null NK cell lines led to G2/M cell cycle arrest, increased apoptosis, and a strong negative selection pressure with progressive elimination of PRDM1-expressing cells, which was enhanced when IL-2 concentration is limiting. We observed a progressive increase in *PRDM1* expression—in particular, *PRDM1*α—in normal NK cells in response to IL-2 and in normal NK cells activated with an engineered NK cell target, K562-Cl9-mb21, suggesting its role in NK cell homeostasis. In support of this role, knockdown of PRDM1 by shRNA in normal NK cells resulted in the positive selection of these cells. We identified *MYC* and *4-1BBL* as targets of PRDM1 in NK cells. Disruption of homeostatic control by PRDM1 may be an important pathogenetic mechanism for NKCL.

NK-cell activation and homeostasis | neoplastic transformation | biotage pyrosequencing | *CCNG1* | *CCNG2*

Natural killer (NK) cell malignancies comprise 1–2% of all non-Hodgkin lymphomas (NHL) (1) and have been classified into aggressive NK cell leukemia (ANKCL) and extranodal NK cell lymphoma of nasal type (ENKCL) by the World Health Organization classification (1). These malignancies are clinically highly aggressive and associated with poor survival (2). The incidence is higher in East Asia and Central and South America compared with other parts of the world (3). Especially at late-stage disease, the malignancies are resistant to chemotherapy, possibly owing to multidrug resistance associated with the expression of P-glycoprotein, a product of the *MDR1* (*ABCB1*) gene (4). Several genome-wide studies identified 6q21 deletion as the most frequent aberration in these tumors (5–8). We observed a combination of deletions, mutations generating truncated, nonfunctional proteins, and promoter methylation of *PRDM1* in the majority of the NK cell lines studied, supporting the role of *PRDM1* as a tumor suppressor gene in this commonly deleted region (9). Interestingly, two recent reports (10, 11) demonstrated frequent mutations of *PRDM1* leading to the formation of nonfunctional proteins in activated B-cell–like diffuse large B-cell lymphoma (ABC-DLBCL), thus implicating *PRDM1* as a tumor suppressor in this subtype of DLBCL. PRDM1 is a transcriptional repressor and a master regulator of the differentiation of B cells into antibody secreting cells (ASC) (12). The repression of *MYC* (13), *SPIB*, *BCL6*, *ID3*, and *PAX5* is required for this function (14). Recently, PRDM1 expression has been detected in CD8+ and CD4+ T cells with the effector phenotype (15, 16), and in CD25+ regulatory T cells (16). Moreover, PRDM1 has been shown to regulate T-cell homeostasis (15, 16). *PRDM1* has two alternative promoters—one generates PRDM1α, which is the functional form of PRDM1, and the other generates a functionally impaired form of PRDM1 (PRDM1β) that lacks the 101 aa at the N terminus (17). Here, we validated the frequent inactivation of PRDM1 in NKCL patient samples predominantly through promoter methylation and demonstrated the tumor suppressor activities of *PRDM1* in NK cell lines. Furthermore, we demonstrated that the knockdown of PRDM1 in normal NK cells promotes their growth.

Results

Evaluation of Copy Number Changes of *PRDM1*. We used the quantitative PCR (qPCR) method on genomic DNA of tumor biopsies to quantify the *PRDM1* copy number. The method was validated by comparing the qPCR results with array CGH (aCGH) data of 6q21 in NK and γδ T-cell lymphoma cell lines. We observed a high concordance (11 of 11, 100%) of the two methodologies (Fig. S1*A*). Because tumor samples contained a varying proportion of nontumor cells, we studied the effect of the stromal content in the tumor biopsies on the qPCR assay by applying it to genomic DNA containing an admixture of DNA from NKYS [an NK cell line with del(6)(q21)] and DNA from human tonsil in predetermined ratios. We found that the qPCR assay was unable to reliably detect the deletion in the presence of 30% or more tonsil DNA (Fig. S1*B*). This level of stromal contamination may cause practical challenges in detecting deletion of *PRDM1* using qPCR or aCGH and FISH would be a useful alternative in this setting if tissues are available. We performed qPCR on the genomic DNA of 18 NKCL cases and observed deletion in 44% (eight of 18) of the cases (Fig. 1*A*), consistent with a previous report showing a 43% (three of seven) deletion rate obtained with BAC aCGH using a smaller set of NKCL cases (9). These results indicate that *PRDM1* deletion is a frequent aberration in primary tumors as well. Our estimate was a minimum approximation, because the qPCR results of two NKCL cases were ambiguous (Fig. 1*A*). The aCGH performed on these two cases was not able to detect 6q21 deletion encompassing *PRDM1*, and therefore the 6q21 status in these cases was regarded as normal. qPCR did not detect deletion in two NKCL cases that were determined to have deletion by aCGH. The aCGH data for NKCL cases was obtained through BAC, Cartes d'Identité des Tumeurs (CIT), or SNP array platforms, and there were several discrepancies between qPCR and the BAC or CIT platforms. In contrast, we observed 100% (six of six) correlation of qPCR and the high-resolution SNP array results.

Promoter Methylation of *PRDM1* in NKCL Cases. We found that *PRDM1*α promoter methylation was associated with repression of *PRDM1*α transcription (9) in malignant NK cell lines. We performed methylation analysis to cross-validate our previous

Author contributions: C.K., J.I., T.W.M., and W.C.C. designed research; C.K. and X.H. performed research; P.G., L.D.L., G.S., and W.Y.A. contributed new reagents/analytic tools; C.K. analyzed data; and C.K. and W.C.C. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1115128108/-/DCSupplemental.

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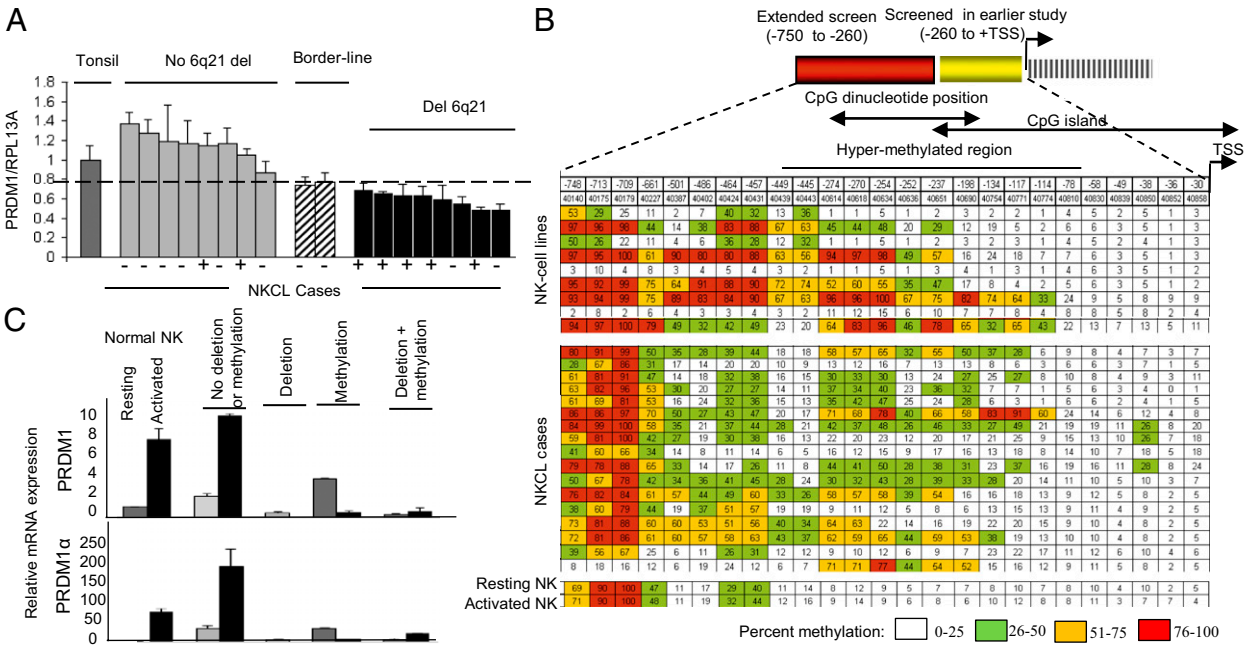

Fig. 1. PRDM1 is silenced through a combination of deletion and promoter methylation. (*A*) NKCL cases with or without deletion is determined by the PRDM1/RPL13A ratio (three replicates), and cases with <0.75 ratio (dashed horizontal line) to that of human tonsil DNA are defined as deleted. The deletion status of the NKCL cases by aCGH is indicated by + and −. (*B*) *PRDM1*α promoter CpG island is hypermethylated in NK cell lines and NKCL cases. Methylation percentage (three technical replicates) of CpG dinucleotides upstream of the *PRDM1*α TSS is indicated as a heat map. Resting and activated NK cells (IL-2 for 7 d) are used as negative control samples. (*C*) qRT-PCR was performed on seven NKCL cases using primers specific for *PRDM1* (α + β) (*Upper*) and *PRDM1*α (*Lower*). *RPL13A* was used for normalization. The deletion or methylation status of PRDM1 for each patient is shown. The expression level is expressed as a ratio of the level for resting NK cells. Data are mean ±SD. Resting human NK cells and activated NK cells derived from 14 d coculture with K562-Cl9-mb21.

findings using a different experimental platform, and extended our analysis to include all available NKCL cases. The region for analysis was extended to include CpG dinucleotides further upstream of the transcription start site (TSS; including 25 CpG dinucleotides between −786 and +1). Nine NK cell lines and 17 NKCL cases were studied along with highly pure (>95% CD56+ CD3− cells) resting and IL-2–stimulated NK cells as control samples. The methylation profile was variable, including a few cell lines and a case that were hypomethylated in regions with methylation in normal NK cells. However, we observed a frequently hypermethylated region compared with normal NK cells encompassing −449 and −114 bp relative to the TSS [12 of 17 (71%) tumor samples, 5 of 9 (56%) NK cell lines] (Fig. 1*B*). Among the 17 cases with deletion and methylation data, 15 of 17 (88.2%) cases demonstrated deletion and/or methylation (three with only deletion, eight with only methylation, and four with both). These results indicate that promoter hypermethylation is a common event and may cooperate with deletions in NK cell malignancies. We observed silencing of *PRDM1*α, as determined with qRT-PCR, in all malignant NK cell lines with promoter hypermethylation except for one NK cell line having methylation in one allele and a deleterious mutation in the other (Fig. S2*B*, *Upper*).

Mutation Analysis of *PRDM1* in NKCL cases. We tested 18 tumor cases and another EBV-negative NK cell line, IMC-1, in addition to the NK cell lines reported previously (9). Unlike NK cell lines, tumor cases did not show any mutation that would result in a truncated protein. We identified two SNPs in three NKCL cases: a missense SNP (position 843 in exon 4, C to G, Asp to Glu; it is a known germline variant rs811925) in one case and a silent SNP (position 2376 G to A, Pro to Pro; it is a germline variant rs1010273) in two cases (Fig. S3 *A* and *B*).

***PRDM1* Expression in NK Cell Tumor Biopsies and IL-2–Activated Primary NK Cells.** The copy number and epigenetic status of NKCL cases correlated well with the *PRDM1* mRNA levels in NKCL cases as determined with qRT-PCR. In general, *PRDM1* expression was low in cases with deletion, methylation, or both (Fig. 1*C*). We used primers (Fig. S2*A*) specific for expression of *PRDM1*α (Fig. 1*C*, *Lower*) or both isoforms (*PRDM1* α + β; Fig. 1*C*, *Upper*). In most instances *PRDM1*α and total *PRDM1* transcript levels were parallel. We had observed up-regulation of *PRDM1*α mRNA in normal NK cells on IL-2 stimulation within a 24-h period (9); now we found that the progressive increase in *PRDM1* expression in primary human NK cells continued for 6 d (Fig. S2*C*).When the *PRDM1*α-specific primers were used for qRT-PCR, the induction of *PRDM1* appeared to be mainly contributed by *PRDM1*α; however, *PRDM1*α mRNA is much lower in resting primary NK cells compared with *PRDM1* (α + β), as reported previously (18). We assayed *PRDM1* expression in primary NK cells obtained from coculture with the K562-Cl9-mb21 cells and observed a robust induction of *PRDM1*α (70-fold) compared with resting NK cells (Fig. S2*D*).

Reconstitution of PRDM1 Leads to Impaired G2/M Cell Cycle Progression and Increases Apoptosis in PRDM1-Null NK Cell Lines. We used the retrovirus murine stem cell virus (MSCV)-internal ribosome entry site (IRES)-GFP (known as pMIG) to reconstitute PRDM1 expression in PRDM1-null NK cell lines (KHYG1, KAI3). We compared the percentage of apoptotic cells in KHYG1 and KAI3 cells transduced with empty vector or PRDM1 using Annexin V staining and observed increased apoptosis in PRDM1-transduced populations of KHYG1 (average = 1.5×) and KAI3 (average = 3.9×) cell lines (Fig. 2 *A* and *B*) 2 d after transduction. Using Hoescht 33342 staining, we observed an increase in the percentage of cells in G2/M phase (4.6% in KHYG1, 7.3% in KAI3) with a concomitant reduction in the percentage of cells in G_o/G_1 (8.8% in KHYG1, 6.2% in KAI3) and S-phases (2.2% in KHYG1, 1.4% in KAI3) in PRDM1-transduced KHYG1 and KAI3 cells on day 3 (Fig. 2 *C* and *D*).

Ectopic Expression of PRDM1 Exerts a Negative Selection Pressure on PRDM1-Null NK Cell Lines in an IL-2 Dose-Dependent Manner. We asked whether reconstitution of PRDM1 expression in PRDM1-

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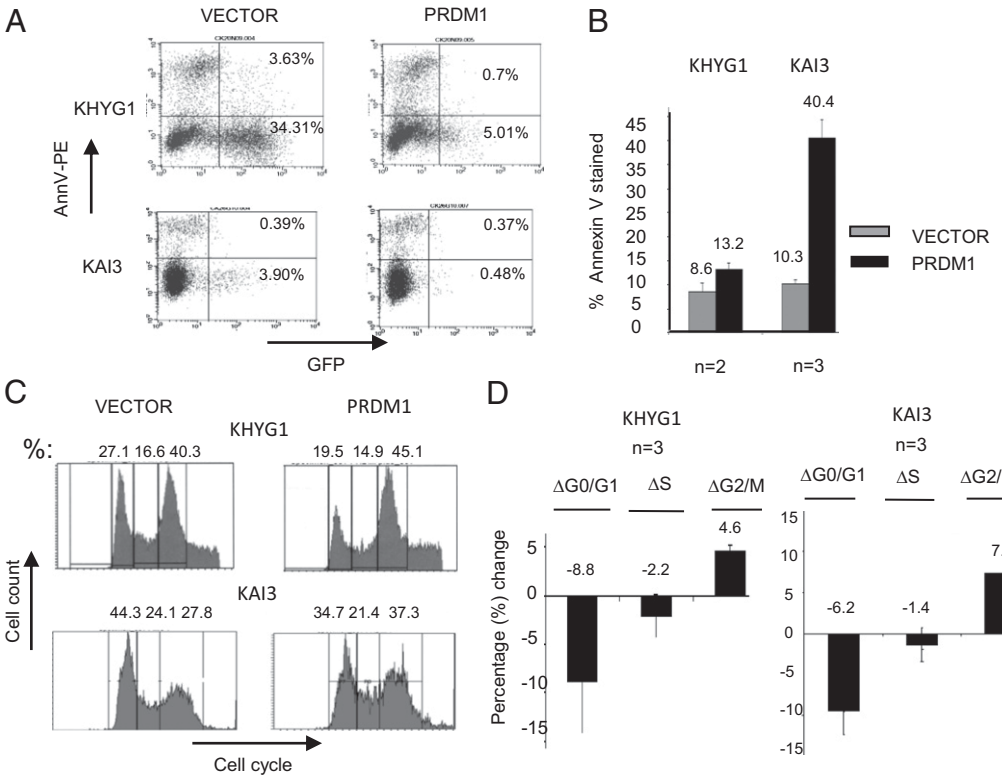

Fig. 2. Reconstitution of PRDM1 in PRDM1-null NK cell lines induces apoptosis and cell cycle arrest. (*A*) PRDM1 induces apoptosis in NK cell lines. Transduced, unsorted NK cells were stained with Annexin V-PE and tested with FACS. The rate of apoptosis was determined by measuring the proportion of Annexin V(+) cells in the GFP(+) population 2 d posttransduction. (*B*) Quantification of the rate of apoptosis in GFP(+) population of vector- or PRDM1-transduced cells. (*C*) Cell cycle profile of vector- or PRDM1 transduced KHYG1 and KAI3 cells. (*D*) The change in each cell cycle phase is calculated as PRDM1 – PMIG 3 d posttransduction.

null NK cell lines will exert a negative selection pressure, as indicated by reduction of the percentage of GFP(+) cells over time in cells transduced with PRDM1 but not those transduced with empty vector . We quantified the percentage of GFP(+) cells in PRDM1-null NK cell lines at different time points (days 2 and 5 for KHYG1 and NK92; days 3 and 6 for KAI3) after transduction with empty vector or PRDM1 and observed a decrease in the percentage of GFP(+) cells only in PRDM1-transduced KHYG1, NK92, and KAI3 cells but not in empty vector-transduced cells (24% in KHYG1, 26% in NK92, and 61% in KAI3; Fig. S4 *A–C*). Next, we tested whether the rate of selective elimination of GFP(+) cells in PRDM1-transduced NK cell lines was influenced by the concentration of IL-2 in the culture medium. In this experiment, the NKYS cell line was used as the negative control, as this NK cell line expresses endogenous PRDM1 protein (9). We cultured the PRDM1-null KHYG1 cells with progressively lower concentrations (100, 75, 50, and 25 IU) of IL-2 and compared the percentage of GFP(+) cells in empty vector- or PRDM1-transduced cells after 2 d and 5 d using FACS (Fig. 3 *A* and *B*). After 2 d of culture, we observed a decrease in the percentage of GFP(+) cells (26.7%, 34.3%, 34.2%, and 39.4% in PRDM1-transduced KHYG1 cells cultured in 100, 75, 50, and 25 IU IL-2, respectively) (Fig. 3*C*, *Left*). After 5 d, we observed a more dramatic decrease in the percentage of GFP(+) cells that is negatively correlated with the IL-2 concentration (73.1%, 76%, 79.4%, and 85.3% in 100, 75, 50, and 25 IU IL-2–treated cells, respectively; Fig. 3*C*, *Middle*). Similarly, we observed a decrease in the percentage of GFP(+) cells (average: 41.2%, 39.9%, 44.5%, and 46.4% for 100,75, 50, and 25 IU IL-2, respectively) in PRDM1-transduced NK92 cells after 3 d of culture. In contrast, we observed a much smaller (overall 35%) IL-2–independent reduction in GFP(+) cells in PRDM1-transduced NKYS cells after 5 d (Fig. 3*C*, *Right*).

Rate of Apoptosis in PRDM1-Transduced NK Cells Proportionately Increases with Progressively Decreasing Doses of IL-2 in the Culture Medium. Next, we asked whether there is an IL-2 dose-dependent increase in the rate of apoptosis in PRDM1-transduced NK cells. PRDM1-transduced NK cells had increased Annexin V staining for all IL-2 concentrations tested (Fig. 3*D*). In addition, we observed a progressive increase in the rate of apoptosis specifically in PRDM1-transduced cells with decreasing doses of IL-2 correlating with the higher rate of negative selection pressure observed in lower IL-2 concentrations (1.08-, 1.24-, and 1.46-fold in 75, 50, and 25 IU IL-2 compared with 100 IU IL-2 treatment, respectively). The inverse correlation between Annexin V staining and the concentration of IL-2 was specific for the GFP(+) population in PRDM1-transduced KHYG1 cells (Fig. 3*E*, *Left*) and was not observed in GFP(+) and GFP (−) populations in vector-only transduced KHYG1 cells or in the GFP(−) population of PRDM1-transduced KHYG1 cells (Fig. 3*E*).

Progressive Elimination of PRDM1-Transduced NK Cells on Long-Term Culture. The percentages of GFP(+) cells in PRDM1 or control vector-transduced KHYG1 and NKYS cells grown with standard (75 IU) IL-2 concentrations were quantified in a time-course experiment. We observed a time-dependent progressive depletion of GFP(+) cells in PRDM1-transduced KHYG1 cells (14.3–0.9%; Fig. S5*A*) and a decrease (37.4–17.1%; Fig. S5*B*) in PRDM1-transduced NKYS cells after 5 wk of culture, but there was no change in the empty vector-transduced KHYG1 (59.5–56.47%) or NKYS (55.9–60.7%) cells, suggesting that a negative selection pressure exerted by the ectopic expression of PRDM1 in NK cells led to the elimination of transduced cells. As an additional control sample, we did not observe any reduction in GFP(+) cells in hTERT-transduced KHYG1 cells in 18 d (Fig. S5*C*). Ectopic expression of *PRDM1* and *hTERT* was confirmed by q-RT-PCR (Fig. S6 *A–C*).

Knockdown of PRDM1 Confers a Growth Advantage to Primary NK Cells. Next, we wanted to test whether knockdown of PRDM1 provides a growth advantage for primary human NK cells. We quantified the percentage of GFP(+) cells at different time points after transduction of PRDM1-expressing NKYS cells and human peripheral blood NK cells derived from the peripheral blood lymphocytes (PBLs) cocultured with K562-Cl9-mb21

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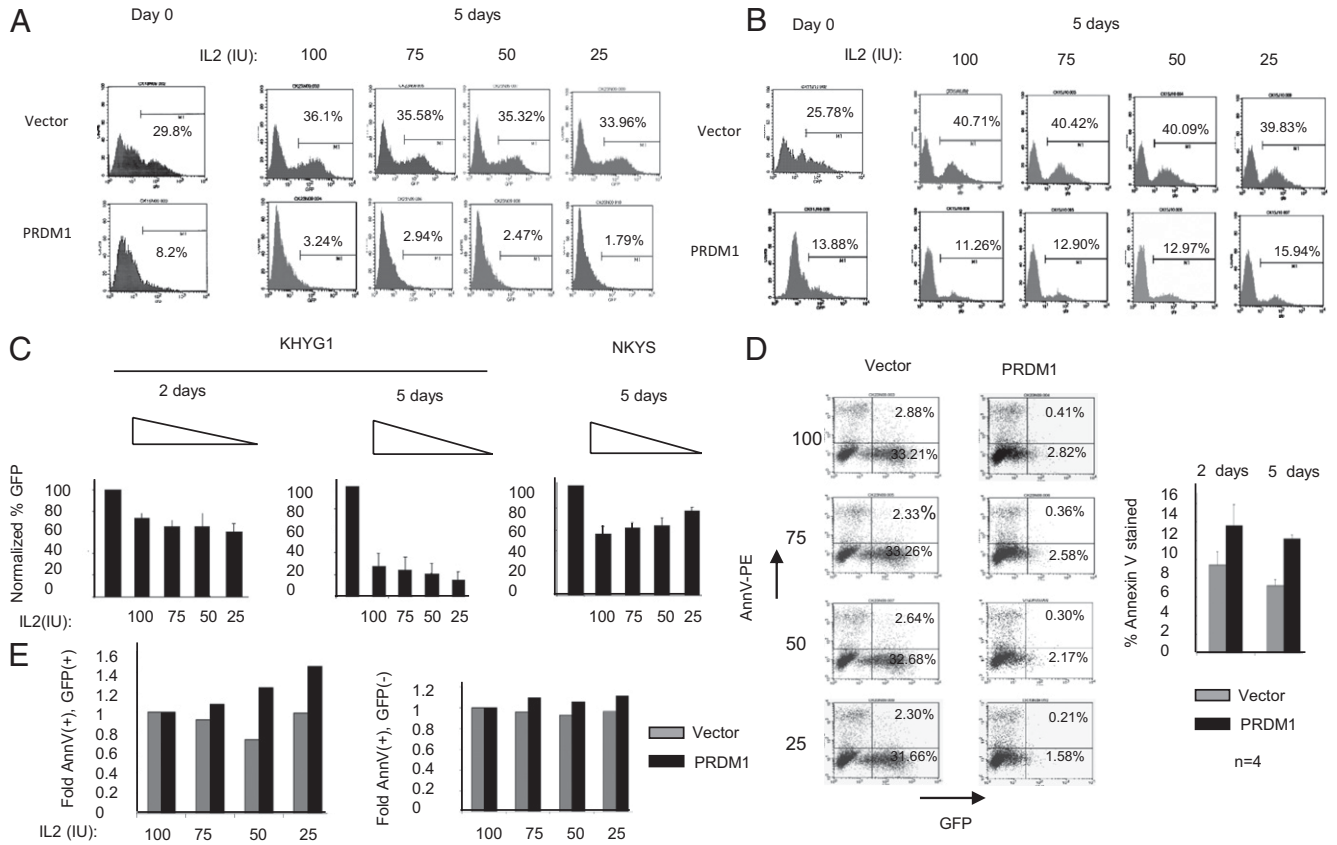

Fig. 3. Reconstitution of PRDM1α exerts negative selection pressure in malignant NK cell lines, which increases with decreasing doses of IL-2 in the culture medium. The FACS profile showing the percentage of GFP(+) cells of the vector- or PRDM1-transduced KHYG1 (*A*) and NKYS cells (*B*) before and after culturing with progressively decreasing doses of IL-2. (*C*) Comparison of the percentage of GFP(+) cells before and after treatment of PRDM1-transduced KHYG1 and NKYS cells with limiting doses of IL-2. Each data point was calculated as follows: [%GFP(PRDM1)]/[%GFP(vector)]. IL-2 posttreatment values were normalized to the values 48 h posttransduction. Data are mean ± SD of two independent experiments. (*D*) Comparison of apoptosis with limiting doses of IL-2 in the presence and absence of PRDM1. PRDM1- or vector-only transduced KHYG1 cells were treated with progressively decreasing doses of IL-2 for 2 or 5 d 48 h posttransduction. (*E*) PRDM1-dependent induction of apoptosis is enhanced with limiting IL-2 concentrations in malignant NK cells.

using empty vector or vector expressing PRDM1 shRNA. We observed a 65% increase in the percentage of GFP(+) cells after knockdown of PRDM1 in NKYS cells when cells at 7 d after transduction were compared with 3 d (Fig. 4*B*). We observed a 90% increase in GFP(+) cells in PRDM1 shRNA-transduced primary NK cells between days 4 and 7 and a 245% increase between days 7 and 10 (Fig. 4*C*). The functionality of the PRDM1 shRNA-mir construct and the knockdown of PRDM1 were shown with the luciferase reporter assay and Western blotting (Fig. S7 *B*, *D*, and *E*).

Altered PRDM1 Expression Is Associated with Changes in Genes Involved in Cell Cycle and Activation in NK Cells. Next, we wanted to test whether ectopic expression of PRDM1 in NK cells alters expression of genes involved in growth by examining a number of known PRDM1 targets. Select targets were evaluated at a later time point in NKYS cells compared with the KHYG1 cells postinfection due to the practical challenges associated with obtaining sufficient quantity of RNA from sorted cells. We observed a decrease in the expression of MYC, *4-1BBL* (*TNFSP9* or *CD137L*), and *TNF*α. There was a corresponding increase in the expression of two negative regulators of the cell cycle, *CCNG1* and *CCNG2*, in KHYG1 cells transduced with PRDM1 (Fig. 5*A*). In contrast, we observed increased expression of *TNF*α, *TNF*β, and *MYC* in *PRDM1* shRNA-transduced NKYS cells compared with the vector-only transduced cells (Fig. 5*B*). We also observed a strong reverse correlation of *MYC* and *PRDM1* mRNA expression levels in IL-2–activated PB NK cells (Fig. 5*C* and Fig. S2*C*, *Lower*).

Discussion

Tumor development is a multistep process often involving cooperation between deregulated expression and/or activation of oncogenes and inactivation of tumor suppressor genes (TSG). Monoallelic deletions are commonly observed in many cancer types, and commonly deleted regions contain potential tumor suppressor genes that may lose function through promoter methylation or mutations in the remaining allele. Given the strong inverse correlation between the *PRDM1*α promoter hypermethylation pattern and the expression levels of *PRDM1*α observed in PRDM1-null NK cell lines and the increase in *PRDM1*α expression after treatment with the DNA demethylating drug 5-aza-2-deoxycytidine (9), promoter methylation may be one of the pivotal mechanisms contributing to the silencing of PRDM1 in NK cells. The fact that the majority of NKCL cases have *PRDM1* promoter hypermethylation suggests that PRDM1 may have been silenced by the combination of deletion and promoter methylation. Deleterious mutations may be infrequent in NKCL cases so that they were not observed in the cases we studied. However, it is also conceivable that there were sequence alterations of *PRDM1* in the NKCL cases that were not detected, as deleterious sequence alterations of *PRDM1* due to aberrant RNA editing were observed in some DLBCL cases (11). Another possibility is that mutations may have been missed because of the contamination of neoplastic NK cells with normal stromal cells. However, this possibility is unlikely, because our gene expression profiling and copy number analysis studies (19, 20) on these cases indicated that the neoplastic NK cells represent the predominant population in the tumor. Therefore, abnormal hypermethylation

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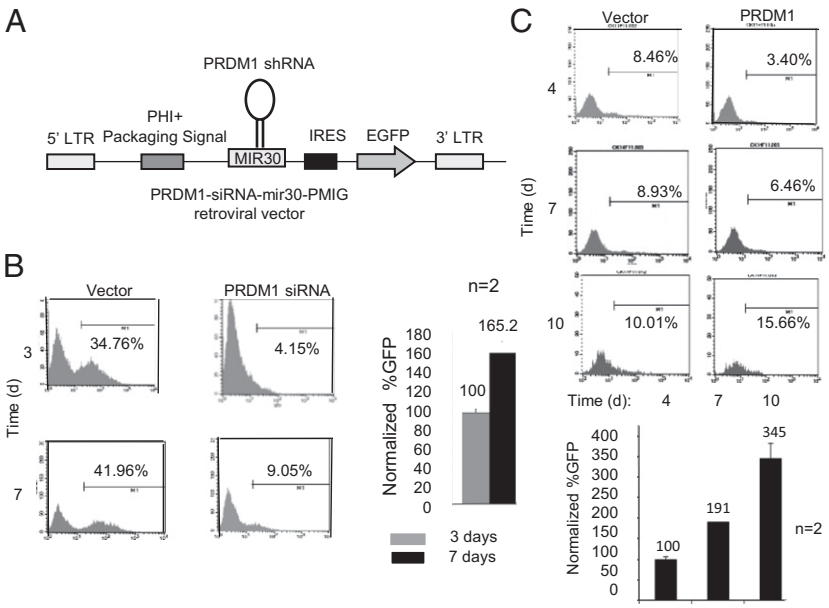

Fig. 4. Knockdown of PRDM1 with shRNA results in the positive selection of human primary NK cells. (*A*) PRDM1 shRNA was PCR cloned inside the miR-30a backbone in MSCV-TMP. The percentage of GFP(+) cells was compared between vector and PRDM1 siRNA-transduced NKYS (*B*) or primary NK cells (*C*) 3 and 7 d (NKYS) or 4, 7, and 10 d (primary NK cells) posttransduction. Data are mean ± SD of two independent experiments.

seemed to account for the vast majority of cases with low PRDM1 expression. We observed an increase in apoptosis and cells in G2/M cell cycle in two PRDM1-null malignant NK cell lines on reintroduction of PRDM1 into the cells; these results provide experimental support for PRDM1 as a TSG in NK cells.

The concomitant increase in expression of two negative cell-cycle regulators and a decrease in *MYC* expression also support the hypothesis that PRDM1 is a tumor suppressor in NK cells. Intriguingly, the *MYC* signature was up-regulated in NKCLs (20). Two known targets of PRDM1 with growth regulatory function in B cells, *MYC* (21) and *4-1BBL* (22), were down-regulated upon expression of PRDM1, suggesting that certain functional role of PRDM1 is conserved in different lymphocyte lineages. Induction of the expression of other direct targets of PRDM1 in NK cells (e.g., *TNF*α and *TNF*β) (18) may cooperate with the reconstitution of MYC expression in the neoplastic transformation of NK cells with silenced PRDM1 expression. Two negative regulators of G2/M cell cycle, CCNG1 and CCNG2 (23), may be indirect targets of PRDM1. In fact, CCNG2 was shown to be repressed by MYC (24).

The progressive increase in apoptosis and negative selection observed when the PRDM1-transduced cells were cultured in decreasing IL-2 concentrations suggests that the tumor suppressor role of PRDM1 in NK cells may be more pronounced when growth-promoting factor is present at a limiting concentration, as is likely to be the case in vivo. PRDM1 may play an important role in the homeostasis of NK cells by preventing excessive NK cell activation and proliferation, and this may be the mechanistic basis for the tumor suppressor action of PRDM1 and the negative selection pressure observed in PRDM1-transduced malignant NK cells. This contention is supported by our findings in human primary NK cells, in which *PRDM1* is up-regulated on activation with IL-2 and proliferation is enhanced upon PRDM1 knockdown. A recent report showed enhanced proliferation of ex vivo-cultured Rag2$^{-/-}$/*prdm1*$^{gfp/gfp}$ mouse NK cells in limiting IL-15 concentrations, suggesting the involvement of PRDM1 in the control of homeostasis of mouse NK cells (25) in agreement with our observations. We have demonstrated up-regulation of *PRDM1*α in response to IL-2 in 24 h in normal NK cells from peripheral blood (Fig. S2*C*, *Upper Left*). We have extended this initial observation and showed that the increase in the transcriptional level of *PRDM1*α continues slowly and progressively in 6 d up to 11-fold, further suggesting that PRDM1 may be part of a negative autoregulatory loop similar to the one observed in T cells (26), controlling the activation and/or homeostasis of NK cells.

Recently, two groups have independently shown PRDM1 to be a tumor suppressor gene in ABC-type DLBCL using in vivo mouse models (27, 28). In ABC-DLBCL, a block in differentiation at the preplasmablast stage of differentiation is postulated to be the pathogenetic mechanism. However, the concomitant

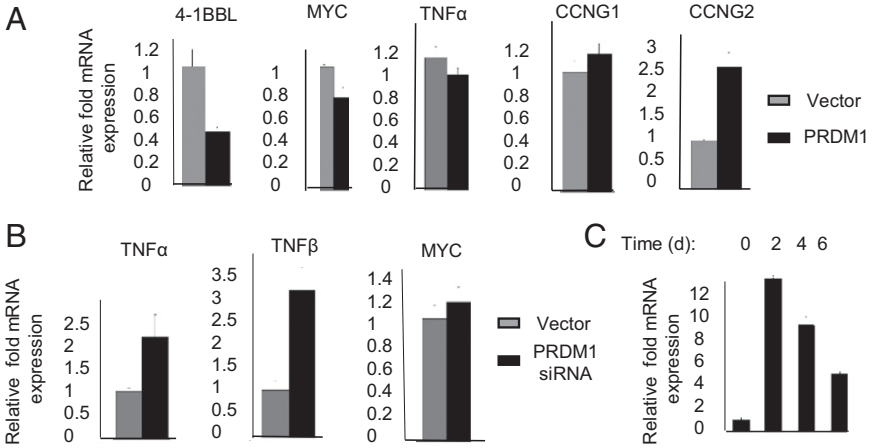

Fig. 5. PRDM1 regulates expression of genes involved in cell cycle and activation in NK cells. (*A*) qRT-PCR results of target genes regulated by PRDM1 in vector- or PRDM1-transduced, GFP-sorted KHYG1 cells 2 d posttransduction. (*B*) qRT-PCR results on vector- or PRDM1 shRNA-transduced, GFP-sorted NKYS cells 6 d posttransduction. (*C*) *MYC* mRNA expression was performed by qRT-PCR on primary NK cells activated by IL-2. *RPL13A* was used for normalization (n = 2).

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failure to repress certain target genes such as *MYC* may also play a role. NK cells may not have as distinct a differentiation stage transition as that from B cells to plasma cells, but there is evidence that PRDM1 is more highly expressed in the most mature population (18). Therefore, our results and published literature suggest that *PRDM1* shares certain functions across lymphocyte lineages and that loss of function of PRDM1 may contribute to the neoplastic transformation of activated B and NK cells through overlapping mechanisms. Intriguingly, we encountered difficulty in reconstituting PRDM1 expression. The challenge of ectopic expression is a common feature of tumor suppressor genes (29) and notably has been observed for PRDM1 in many instances, even with the inducible constructs in GC B-cell–derived cell lines (22). This phenomenon was more obvious when we compared the mean fluorescence intensity (MFI) of NK cells transduced with vector, PRDM1, and hTERT (Fig. S8*B*). We observed lower MFI and lower numbers of GFP(+) cells with inducible PRDM1 constructs [i.e., PRDM1-ER and PRDM1a-TREmiR30-PIG (TMP) even before induction, suggesting leaky transgene expression and selective disadvantage of PRDM1 expression, even at low expression levels.

In summary, we performed both functional and correlative studies on PRDM1 in normal and neoplastic NK cells and provide strong evidence that PRDM1 is a tumor suppressive gene in NKCLs. Silencing of PRDM1 in NKCL cases is mainly through cooperation of 6q21 deletion and promoter CpG island hypermethylation. Reintroduction of PRDM1 into NK cell lines lacking its expression enhanced apoptosis and impaired G2/M cell cycle progression, and these cells are negatively selected in cell culture. Conversely, knocking down PRDM1 expression provides a growth advantage to activated PB NK cells. PRDM1 may share important target genes in B and NK cells, such as *MYC*, and identifying the essential targets will lead to further understanding of its pathogenetic mechanisms.

Materials and Methods

Patient Material and Cell Line. The characteristics of NK cell tumor cases and NK and γδ T-cell lines included in this study have been reported previously (19, 20) and are summarized in Tables S1 and S2. Genomic DNA extraction was performed with the standard phenol/chloroform method (9). The four cell lines, KHYG1, KAI3, NK92, and NKYS, used for transduction studies were cultured in RPMI-1640 (Gibco-Invitrogen) supplemented with 10% FCS; penicillin G (100 units/mL) and streptomycin (100 μg/mL); and 75 IU IL-2 (R&D Bioscience) at 37 °C in 5% CO_2.

Primary NK Cell Isolation and Culture. Normal NK cells from PBLs of healthy donors were isolated through negative selection using an NK Cell Isolation Kit (Miltenyi Biotec Inc.). Purity of NK cells was validated with CD56-APC (Miltenyi Biotec Inc.) and CD3-PE (Miltenyi Biotec Inc.) staining using FACSCalibur. Isolated NK cells were cultured as described previously with 200 IU IL-2 (R&D Bioscience).

Full experimental procedure of PRDM1 copy number variation, mRNA expression, promoter methylation analysis, exon/exon–intron junction sequencing, cell cycle and apoptosis assays, PRDM1 reconstitution experiments, PRDM1 shRNA design and knockdown in NK cells, luciferase assay, and Western blot are presented in *SI Materials and Methods*.

Note Added in Proof

While this paper was under review, a paper describing similar findings on PRDM1 was published (30).

ACKNOWLEDGMENTS. We thank Dr. Dean A. Lee for the K562-Cl9-mb21 cell line; Dr. Norio Shimuzu for four NK-cell lines (SNK-1, SNK10, SNK-6, NK-YS) and the γδ T-cell lymphoma lines (SNT13, SNT15, and SNT8); Dr. I-Ming Chen for the IMC-1 cell line; Drs. Yulei Shen and Zhongfeng Liu (University of Nebraska Medical Center DNA Microarray Core Facility) for technical assistance; and Dr. Runqing Lu and Himabindu Rhamachandrareddy for helpful suggestions. YT and NK-92 were obtained from the German Collection of Microorganism and Cell Culture (GCMCC) (DSMZ, Braunschweig, Germany). KHYG1, KAI3 cell lines were obtained from the Health Science Research Resource (Osaka, Japan). This work was supported in part by Lymphoma SPORE P50CA136411-01(NC1), National Cancer Institute Grant 5U01/CA114778, Eppley Cancer Institute Core Grant CA36727, and Council/General Research Fund of Hong Kong Grant HKU 776309M. The University of Nebraska Medical Center Microarray Core Facility is supported partially by National Institutes of Health Grant P20 RR016469 from the Nebraska IDeA Network of Biomedical Research Excellence Program of the National Center for Research Resources.

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