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RUNX3 Promoter Hypermethylation Is Frequent in Leukemia Cell Lines and Associated with Acute Myeloid Leukemia inv(16) Subtype

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Summary

Correlative and functional studies support the involvement of the RUNX gene family in hematological malignancies. To elucidate the role of epigenetics in RUNX inactivation, we evaluated promoter DNA methylation of *RUNX1*, *2*, and *3* in 23 leukemia cell lines and samples from acute myeloid leukemia (AML), acute lymphocytic leukemia (ALL), and myelodysplatic syndromes (MDS) patients. *RUNX1* and *RUNX2* gene promoters were mostly unmethylated in cell lines and clinical samples. Hypermethylation of *RUNX3* was frequent among cell lines (74%) and highly variable among patient samples, with clear association to cytogenetic status. High frequency of *RUNX3* hypermethylation (85% of the 20 studied cases) was found in AML patients with inv(16)(p13.1q22) compared to other AML subtypes (31% of the other 49 cases). *RUNX3* hypermethylation was also frequent in ALL (100% of the 6 cases) but low in MDS (21%). In support of a functional role, hypermethylation of *RUNX3* was correlated with low levels of protein, and treatment of cell lines with the DNA demethylating agent decitabine resulted in

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Competing interest

The authors declare no competing financial interests.

Author contribution

MRHE, SM, CBR, HY, KK and ZF performed the research. MRHE, SM, and GGM designed the research. MRHE, SM, CDD, SAP, and GGM analysed the data. YW, WS, KC, GB, and HK contribute with acquisition of clinical samples and reagents. MRHE, SM, CDD, ZSB and GGM wrote the manuscript.

mRNA re-expression. Furthermore, relapse-free survival of non-inv(16)(p13.1q22) AML patients without *RUNX3* methylation was significantly better (p=0.016) than that of methylated cases. These results suggest that *RUNX3* silencing is an important event in inv(16)(p13.1q22) leukemias.

Keywords

acute myeloid leukemia; *RUNX3*; DNA methylation; gene expression; inv(16)(p13.1q22)

Introduction

The RUNX family of transcription factors represents the DNA binding α-chain partners of the heterodimeric core binding factor (CBF) complex (Blyth *et al*, 2005). Each of the RUNX proteins, RUNX1 (AML1), RUNX2, and RUNX3 (AML2) can form heterodimers with CBFβ. In the CBFB-MYH11 subtype of human acute leukemia, the chromosomal translocation resulting in an inversion in chromosome 16 [inv(16)(p13.1q22), referred only as inv(16) in the remaining of this report] encodes a chimeric protein in which CBFβ is fused to smooth muscle myosin heavy chain (*MYH11*) (Liu *et al*, 1993). Although the exact mechanism of leukemogenesis by this chimera is unknown, it is thought that CBFB-MYH11 sequesters RUNX1 in the cytoplasm and antagonizes its normal function (Shigesada *et al*, 2004). Although the role of RUNX1 in hematopoiesis has been previously well-established (Okuda *et al*, 1996), recent data have indicated that the RUNX3 may also play a key role in the development of human acute leukemias (Cheng *et al*, 2008).

DNA hypermethylation of the *RUNX3* promoter and gene downregulation have been reported for human solid tumors (Kim *et al*, 2004), including colon (Silva *et al*, 2013; Subramaniam *et al*, 2009), bladder (Kandimalla *et al*, 2013; Kim *et al*, 2004), stomach (Fan *et al*, 2011; Imamura *et al*, 2005), and lung (Yanagawa *et al*, 2007) cancers. In support of this gene's function as a tumor suppressor, mice lacking Runx3 develop gastric epithelial hyperplasia and tumors as a result of stimulated proliferation and suppressed apoptosis in epithelial cells (Li *et al*, 2002). In leukemia, several studies reported downregulation of *RUNX3* expression in inv(16) AML. Debernardi *et al*. characterized 28 patients with AML using gene expression profiling and found that *RUNX3* was downregulated in patients with inv(16) AML and overexpressed in patients with acute promyelocytic leukemia (t15;17) (Debernardi *et al*, 2003). Two later independent studies also using gene expression profiling confirmed the downregulation of *RUNX3* in inv(16) AML (Gutierrez *et al*, 2005; Sun *et al*, 2007). The role of *RUNX3* in hematopoiesis has been characterized in zebrafish models in which Runx3 inhibition led to a decline in the number of mature blood cells (Crosier *et al*, 2002). Thus, the role of RUNX3 in tumorigenesis, its downregulation in hematopoietic cells, and the finding that RUNX3 is involved in hematopoiesis in animal models all suggest that this gene may contribute to the development of leukemia in humans.

Given the decreased gene expression of *RUNX3* and prior data showing no somatic mutations in this gene (Otto *et al*, 2003), we sought to analyze the promoter methylation status of this gene as well as its two other family members, *RUNX1* and *RUNX2*, in patients with AML. We found that *RUNX3* was hypermethylated in the majority of patients with

AML inv(16). *RUNX1* and *RUNX2* hypermethylation were rare, and *RUNX3* methylation was low in non-inv(16) AMLs.

Material and Methods

Cell lines and AML patient samples

Eleven human leukemia cell lines of myeloid origin (K562, BV173, HL60, NB4, THP1, U937, ML1, OCI-AML3, HEL, MOLM13, and KBM5R) and 12 of lymphoid origin (MOLT4, Jurkat, Peer, T-ALL1, CEM, J-TAG, BJAB, RS4, ALL1, Raji, REH, and Ramos) were used in this study. All cell lines were obtained from the American Type Culture Collection and were cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gemini Bio-Products, West Sacramento, CA, USA) and penicillin–streptomycin (Invitrogen, Carlsbad, CA). Cell suspensions from bone marrow aspirate specimens from patients with AML, MDS, and ALL prior to any therapy were obtained from established tissue blocks at The University of Texas MD Anderson Cancer Center. Peripheral blood samples were obtained from 4 healthy volunteers, and CD34+ cells were obtained from another 4 individuals. All samples from healthy donors were collected using Ficoll-Paque density centrifugation.

DNA extraction and bisulfite modification

DNA was extracted from leukemia cell lines and samples from patients and healthy volunteers using standard phenol-chloroform methods. DNA was subsequently treated with sodium bisulfite as previously described (Estecio *et al*, 2006). Briefly, 2 μg of genomic DNA from each sample was denatured in 0.2 M NaOH at 37°C for 10 min and then incubated with 3M sodium bisulfite at 50°C for 16 hours. DNA was then purified using the Wizard cleanup system (Promega, Fitchburg, WI), and desulfonated with 0.3 M NaOH at 25°C for 5 min. DNA was precipitated with ammonium acetate and ethanol, washed with 70% ethanol, dried, and resuspended in water.

Methylation analysis with pyrosequencing

PCR primers for the CpG island region overlapping the proximal promoters (P2) of *RUNX1*, *RUNX2*, and *RUNX3* were designed using Biotage's PSQ primer design software (Biotage AB, Uppsala, Sweden). Optimal annealing temperatures for each of these primers were tested using gradient PCR. PCR conditions and primers are presented in Table 1. PCR reactions were performed in a total volume of 20 μl, and the entire volume was used for each pyrosequencing reaction. Briefly, PCR product purification was done with streptavidin-sepharose high-performance beads (GE Healthcare Life Sciences, Piscataway, NJ), and co-denaturation of the biotinylated PCR products and sequencing primer (3.6 pmol/reaction) was conducted following the PSQ96 sample preparation guide. Sequencing was performed on a PSQ HS 96 system with the PSQ HS 96 SNP reagents kit (Biotage AB) according to the manufacturer's instructions. The degree of methylation was calculated using the PSQ HS 96A 1.2 software.

Immunohistochemistry of leukemia patient-derived samples

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections of bone marrow biopsy specimens. Sections were de-paraffinized, re-hydrated, and submitted to heat-induced epitope retrieval. Samples were incubated with an anti-RUNX3 rabbit antibody or a negative control rabbit antibody (AML2 a400 affinity-purified antibody kindly provided by Kun-Sang Chang) at a dilution of 1:2000 at room temperature (RT) in a dark humidified chamber for 60 minutes. Detection of the primary antibody was achieved with the EnVision+ system (DakoCytomation, Denmark) containing secondary antibodies conjugated to a horseradish peroxidase complex (HRP). Slides were incubated at RT in a dark humidified chamber for 30 min and were developed with the chromogen 3,3'-diaminobenzidine (DAB)/H_2O_2 (DakoCytomation). Slides were counterstained with hematoxylin, dehydrated, mounted, and cover-slipped.

Treatment with 5-aza-2'deoxycitidine

Seven leukemia cell lines (Raji, HL60, Molt4, ALL1, Jurkat, RS4, and PEER) were treated with the hypomethylating agent decitabine (5-aza-2'-deoxycytidine) to study the effects of epigenetic modulation. Cells were plated at low density 6 – 8 hrs before treatment with decitabine at concentrations of 1, 3, 5, and 10 μM for 3 days. Control samples were treated with dimethyl sulfoxide (DMSO). Media containing decitabine or the same volume of vehicle were changed every 24 hours. Total cellular RNA was extracted with Trizol (Invitrogen) according to the manufacturer's protocol.

Analysis of gene expression with real-time PCR

Three μg of total RNA was used for reverse transcription (RT) reactions. RT reactions were performed using the Moloney murine leukemia virus RT enzyme (Invitrogen) according to the manufacturer's protocol. Quantitative PCR reactions were performed using inventoried TaqMan gene expression assays for *RUNX3* and *GAPDH* and TaqMan universal PCR master mix (Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. Experiments were performed in triplicate for each data point. The signal of *RUNX3* was normalized to the endogenous reference *GAPDH* to obtain the relative threshold cycle and then related to the CT of the control to obtain the relative expression level of the target gene.

Statistical analysis

DNA methylation and clinicopathogical values were compared between groups using the non-parametric Mann-Whitney U test. Survival curve comparison was done with the Log-rank (Mantel-Cox) test. Patients that did not respond to therapy were excluded from the relapse fee survival (RFS) analysis. All calculations were done in GraphPad software (GraphPad Software, Inc., La Jolla, CA).

Results

RUNX3 is frequently hypermethylated in leukemia cell lines

We investigated the DNA methylation status of the *RUNX1*, *RUNX2*, and *RUNX3* gene promoters (Fig. 1A) using bisulfite PCR followed by pyrosequencing in 23 leukemia cell

lines representing AML, CML, and MDS diseases (Table I). Hypermethylation (methylation density equal or above 15%) of *RUNX1* and *RUNX2* was rare in these cell lines; *RUNX1* was not hypermethylated in any of the studied samples, and *RUNX2* was hypermethylated in only 2 cell lines (Raji and B-Jab). In contrast, we found that the *RUNX3* promoter was hypermethylated in 17 (74%) of the cell lines (Fig. 1B). Interestingly, we observed a trend toward higher frequency of hypermethylation of *RUNX3* in cell lines of myeloid (90%) compared to lymphoid (57%) origin (Fig. 1C). The only myeloid cell line with unmethylated *RUNX3* was THP1. Whether there was preferential methylation of *RUNX3* within disease subgroups could not be determined due the small number of cell lines per disease.

RUNX3 can be reactivated by treatment with 5-aza-2'-deoxycytidine

Because demethylating agents like decitabine are efficient in treating leukemias, particularly AML and MDS, we assessed whether treatment with decitabine could elevate the transcription of *RUNX3*. We first screened 7 leukemia cell lines (Jurkat, RS4, Raji, PEER, ALL1, HL60, and Molt4) for *RUNX3* expression using real-time PCR and found that its expression was low to undetectable regardless of *RUNX3* methylation status (Fig. 2A). Next, we treated these cells with various doses of decitabine (1 μM, 5 μM, and 10 μM) or only vehicle for 4 days and measured the expression of *RUNX3* again. An increase in transcripts (2-fold and above) was seen for each cell line, with the exception of Jurkat and PEER, and an increase in expression 5-fold and higher than baseline was seen for Raji and HL60. Significant decreases in *RUNX3* methylation were observed in Molt4 cells after decitabine treatment (decreased from 60% methylation without treatment to 30% after 10 μM decitabine). Despite the increase in gene expression, *RUNX3* methylation was unchanged in HL60 (30% methylation before and after exposure to decitabine).

RUNX3 hypermethylation is associated with cytogenetic status in AML

Because of the high frequency of *RUNX3* expression in myeloid leukemia cell lines, we studied a larger group of clinical samples composed of AML, ALL, and MDS clinical samples (Table II). In total, 102 samples were evaluated by bisulfite modification followed by pyrosequencing, including 8 control samples (4 whole bone marrow and 4 CD34+ bone marrow cells from healthy donors). *RUNX3* promoter methylation was below 15% in normal samples, and hypermethylation was found in 32/69 AML samples (46%), 4/19 MDS samples (21%), and 6/6 ALL samples (100%) (Fig. 3A). Of the 69 AML samples, 20 were classified as inv(16) AML, and 49 were other types of AML. In the AML inv(16) samples, 85% were hypermethylated at the *RUNX3* promoter region, whereas only 31% of the other AML subtypes were hypermethylated (Fig. 3B). We also evaluated DNA methylation of *RUNX1* and *RUNX2* in a subgroup of these samples (66 samples for *RUNX1* and 72 for *RUNX2*) with the same assays and found that, as in cell lines, these genes are almost universally unmethylated; with the exception of a single AML case, all studied samples lacked promoter methylation (data not shown).

A second validation group composed of 16 RUNX1-RUNX1T1 t(8;21) AML samples was evaluated for *RUNX3* methylation, and again we observed only rare hypermethylation (2 cases, 12.5%). Four cases had samples collected both at time of admission and at one or two later timepoints after treatment for the disease. In the 2 hypermethylated cases, we observed

loss of *RUNX3* methylation after treatment. Four new inv(16) AML cases from patients admitted to the hospital after the initial screening for *RUNX3* methylation were included with this validation, and in agreement with the previous findings, these showed frequent *RUNX3* hypermethylation (2 cases).

RUNX3 protein levels are reduced in AML samples and correlated with promoter hypermethylation

We next sought to verify the expression status of *RUNX3* in AML samples. Using immunohistochemistry, we investigated the presence of *RUNX3* protein in eight AML samples with variable DNA methylation status and one normal bone marrow sample (Fig. 4A). Except for one case with 90% of cells positive for RUNX3, all tested AML samples showed a decrease in the number of RUNX3-positive cells (ranging from 1% to 70% positive cells). The decrease in RUNX3 positivity was generally correlated with the DNA methylation density in each case (Fig. 4B), although there were one case with low promoter DNA methylation and low RUNX3 protein expression.

Patients without RUNX3 methylation have longer relapse-free survival

We were able to match clinicopathological information for 16 hypermethylated and 32 non-methylated non-inv(16) AMLs. Using nonparametric tests, we did not observe differences in age or white blood cell and platelet counts (Table II). A significant difference was observed for relapse-free survival (Fig. 5B), with non-methylated *RUNX3* cases showing longer relapse-free survival than methylated cases (p=0.015, Log-rank test). It is important to note that in this analysis we excluded 26 patients that did not respond to therapy. However, overall survival did not differ between the groups (Fig. 5A).

We could not accurately compare clinicopathological features in inv(16) patients because of the low number of non-methylated *RUNX3* cases (only 3 with available information versus 13 hypermethylated cases). Nevertheless, we observed a tendency for better both overall survival and relapse-free survival for patients with unmethylated *RUNX3* (Figs. 5C and 5D). Other significantly different features, such as age, were unreliable due to the sample size.

Discussion

In this study, we have characterized the methylation status of the *RUNX1*, *RUNX2*, and *RUNX3* genes in human myeloid leukemias. Our results show that promoter methylation of the *RUNX3* gene and downregulation of *RUNX3* expression occurs almost universally in inv(16) AMLs, and in cell lines, *RUNX3* repression can be reversed by treatment with the hypomethylating agent decitabine. Furthermore, *RUNX3* hypermethylation was correlated with shorter relapse-free survival in a limited cohort.

The essential function of *RUNX1* in normal hematopoiesis as well as in acute leukemia, specifically the t(8;21) AML M2 subset, has been well characterized (Downing *et al*, 1993; Lo Coco *et al*, 1997; Okuda *et al*, 1996; Peterson and Zhang 2004). *RUNX1* knockout is embryonically lethal in mice, and the resultant embryos show an absence of hematopoietic cell development. In contrast, information on RUNX3 function in leukemia is comparatively limited, and several studies of the *RUNX3* gene have mostly focused on its role as a tumor

suppressor gene in gastric cancer. For example, it has been shown that *RUNX3* knockout mice show a phenotype of gastric epithelial hyperplasia (Li *et al*, 2002).

Some studies suggest that *RUNX3* may have a complementary role to *RUNX1* in acute myeloid leukemia. Double-knockout *Runx1*;*Runx3* mice present a complex phenotype that includes bone marrow failure and, in a subset of animals, signs of myeloproliferative disease (Wang *et al*, 2014). RUNX3 also regulates RUNX1 (Brady *et al*, 2009), which highlights the intricate interplay between RUNX family members.

Studies using cDNA microarray analysis of AML samples have identified *RUNX3* downregulation, and in some studies, this was more prominent in the formally called M4Eo subset (Debernardi *et al*, 2003; Gutierrez *et al*, 2005). However, Otto et al. previously showed that no somatic mutations could be identified in the *RUNX3* gene in AML patients (Otto *et al*, 2003). We therefore hypothesized that *RUNX3* expression in AML may be regulated epigenetically via DNA methylation. Our study showed that the *RUNX3* promoter was hypermethylated in specific subsets of AML, specifically inv(16) AML. This finding differs from those of Cheng et al., who found that RUNX3 expression in AML was not mediated by promoter hypermethylation (Cheng *et al*, 2008). We propose that this difference in results may be due to population characteristics because their study was mostly composed of childhood leukemias and fewer inv(16) cases than we studied.

Scarce information is available regarding the prognostic value of abnormal expression of *RUNX3* in leukemia. Lacayo et al. found that *RUNX3* overexpression among pediatric patients with AML with mutated FLT3 had an inferior prognosis, but in patients with wild-type FLT3, RUNX3 expression had no predictive value for survival (Lacayo *et al*, 2004). In contrast, Cheng et al. showed that high *RUNX3* expression among childhood AML cases was associated with a shortened event-free survival regardless of their FLT3 status (Cheng *et al*, 2008). The patient cohort in our study is composed exclusively of adults, and we observe a trend for longer relapse-free survival among the *RUNX3* unmethylated subset. However, these data are limited to non-inv(16) patients owing to the rarity of unmethylated *RUNX3* cases among the inv(16) subtype. Also, expression data are not available for the group we studied, so direct comparisons to other studies was not possible. It is possible that the trend we observe is not directly related to lack of RUNX3 protein, but rather, that the value of its hypermethylation is a marker of dysregulation of transcriptional programs (given the known interplay between hematopoiesis and RUNX factors) or that *RUNX3* is one of several genes concomitantly hypermethylated in leukemia. In respect to the latter, high frequency of methylated genes has been associated with worse survival in leukemia, T-ALL (Roman-Gomez *et al*, 2005), and MDS (Shen *et al*, 2010).

In summary, our results suggest that epigenetic dysregulation of *RUNX3* is likely an important target in the molecular pathway of leukemogenesis in core binding factor leukemia, and future studies should be dedicated to further characterize the role of RUNX3 in AML.

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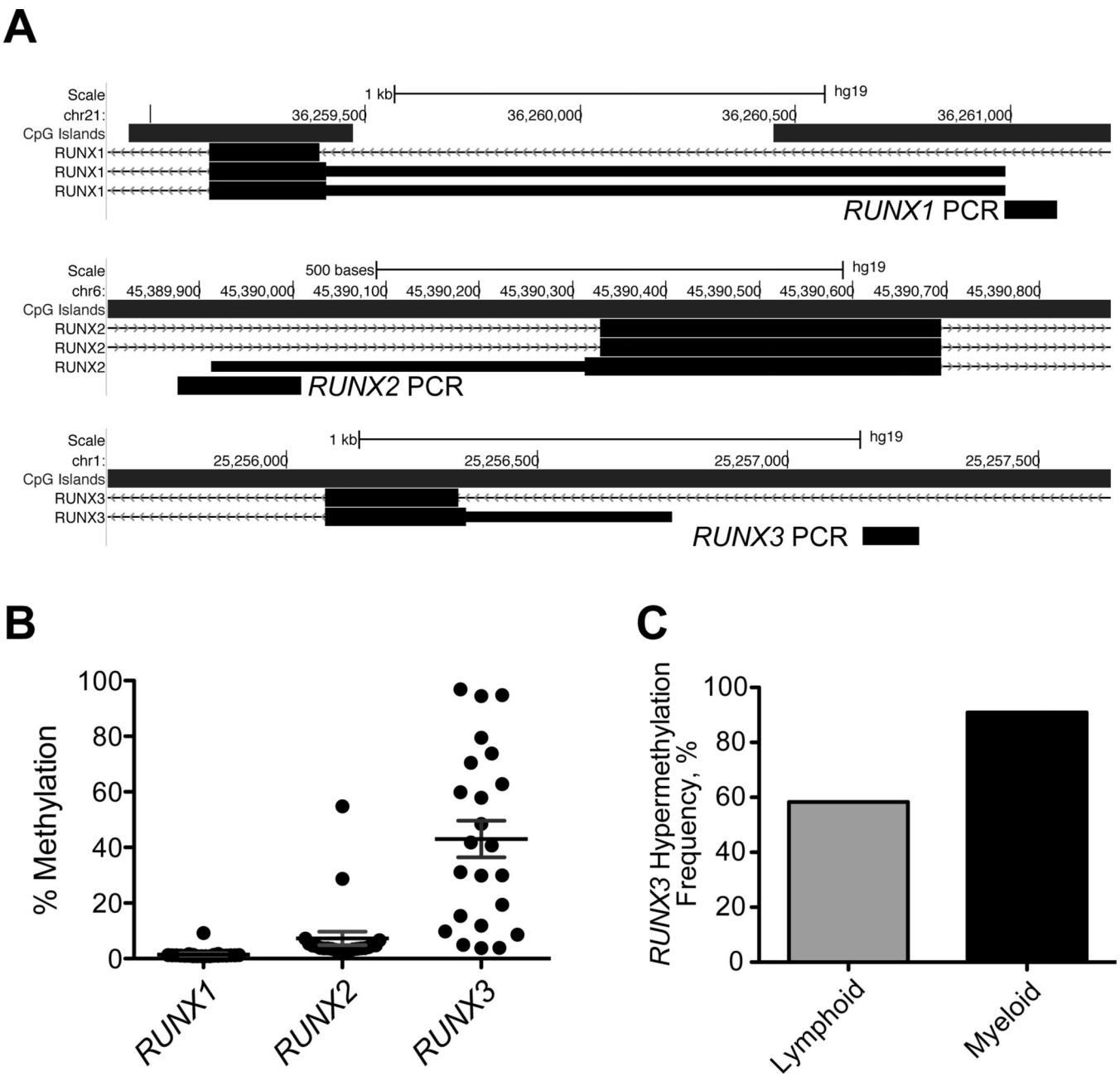

Figure 1.

Gene promoter DNA methylation analysis of *RUNX1*, *RUNX2*, and *RUNX3* in leukemia cell lines. (**A**) Schematic representation of bisulfite PCR primers for downstream pyrosequencing analysis. The generated PCR amplicons were within −500bp to +200bp from the transcription start site (TSS) of the alternative promoter (P2) of each gene, and the methylation density of 2 to 3 CpG sites per amplicon was quantified and averaged. (**B**) DNA methylation density of *RUNX1*, *RUNX2*, and *RUNX3* in 23 leukemia cell lines. Note that only *RUNX3* shows frequent promoter DNA hypermethylation (defined as 15% measured DNA methylation and above). (**C**) Differential frequency of *RUNX3* promoter DNA hypermethylation in lymphoid versus myeloid leukemias.

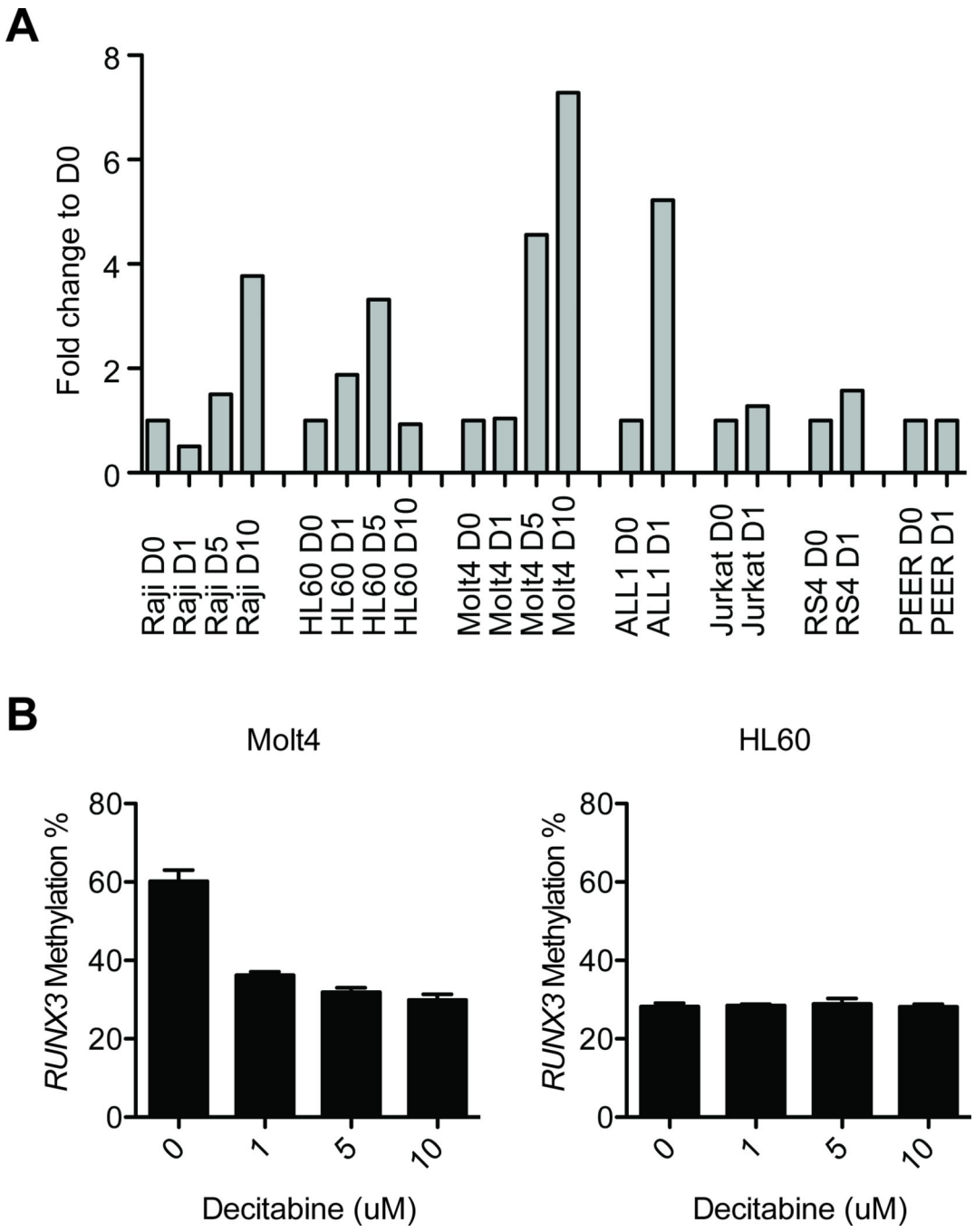

Figure 2.

RUNX3 reactivation after treatment with a demethylating agent. (**A**) Fold change in RUNX3 expression in 7 leukemia cell lines after treatment with various doses of decitabine (D1=1 μM; D5=5 μM; D10=10 μM) or vehicle (D0) for 3 days. An increase in expression over 2-fold was observed for all cell lines treated with high-dose decitabine (Raji, HL60, and Molt4) and in only ALL1 among the cell lines treated with low-dose decitabine. (**B**) *RUNX3* promoter demethylation after treatment with decitabine.

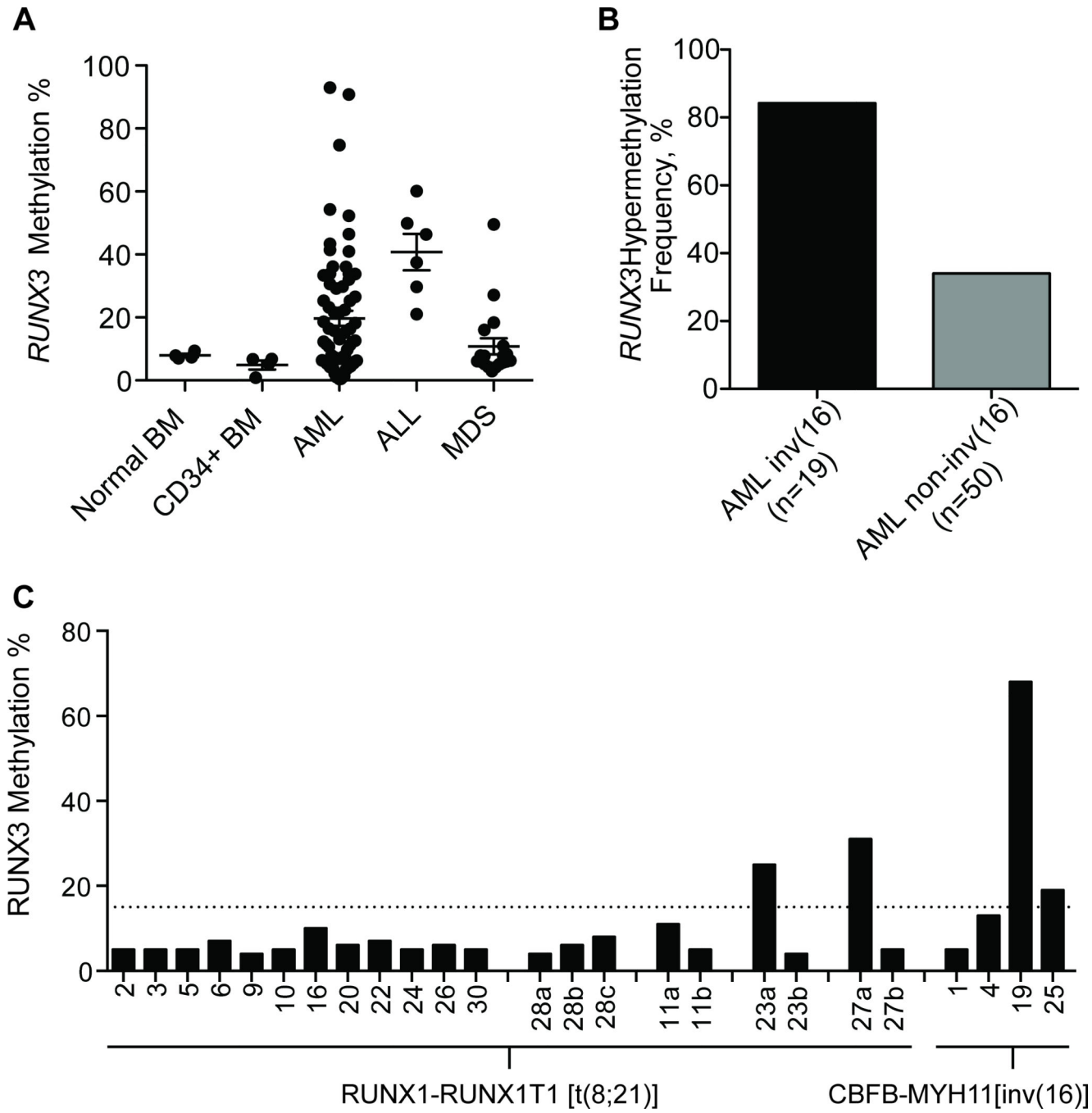

Figure 3.

RUNX3 promoter DNA methylation in clinical samples. (**A**) DNA methylation density of the *RUNX3* promoter in 8 control individuals (4 normal bone marrow [BM] and 4 CD34+ BM), 69 acute myeloid leukemia (AML), 6 acute lymphocytic leukemia (ALL), and 19 myelodysplastic syndromes (MDS) patient samples. (**B**) Differential frequency of *RUNX3* promoter DNA hypermethylation in inv(16) versus non-inv(16) AML subtypes. The inv(16) AML group had a 2-fold higher frequency of *RUNX3* hypermethylation compared to other AML subtypes. (**C**) *RUNX3* methylation in the RUNX1-RUNX1T1 [t(8;21)] subtype. The

letter “a” after the patient number indicates sample collection at admission, and “b” and “c” are post-treatment samples. *RUNX3* methylation is infrequent in this group, and it is decreased after treatment. Additional inv(16) samples were evaluated (CBFb-MyH11), and *RUNX3* was hypermethylated in half of cases.

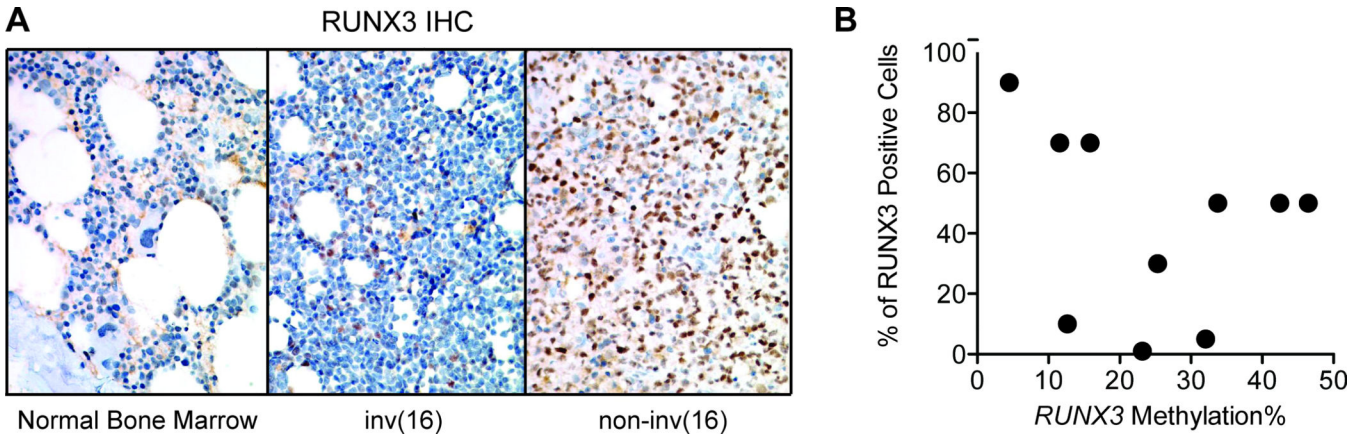

Figure 4.

Protein expression of RUNX3 in selected AML samples. (**A**) Immunohistochemical staining for RUNX3 protein (in brown) in a normal bone marrow sample (left panel), an inv(16) AML case (middle panel), and an non-inv(16) AML case (right panel). (**B**) Correlation of percent of RUNX3-posivitive cells (y-axis) with DNA methylation (x-axis). The percentage of RUNX3-positive cells decreased with increased promoter DNA methylation.

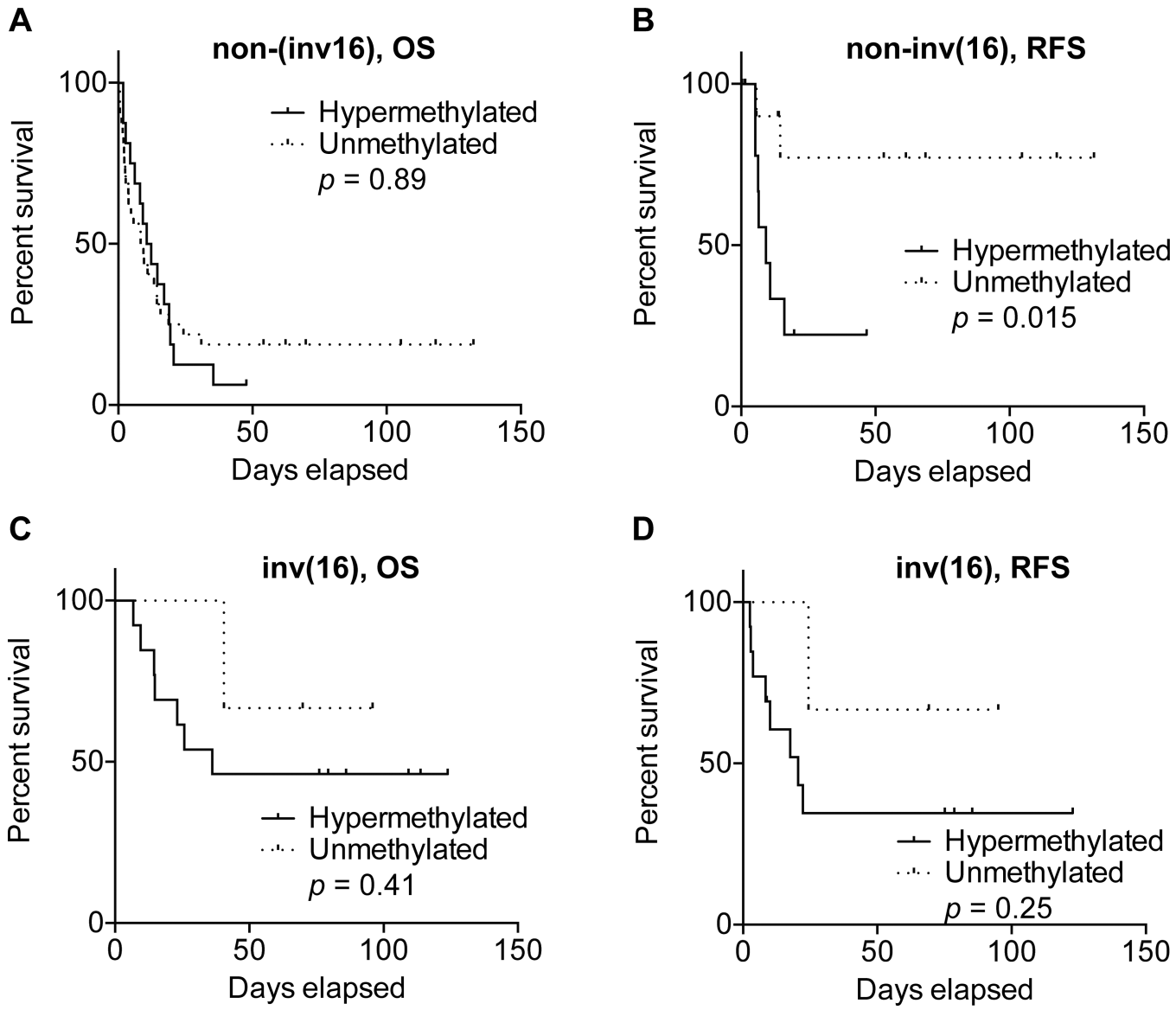

Figure 5.

Survival analysis of AML cases categorized by RUNX3 methylation status. (**A**) Non-inv(16) AMLs have poor overall survival that does not differ between RUNX3 hypermethylated (solid line) and unmethylated groups (dotted line). (**B**) Relapse-free survival was significantly longer in unmethylated RUNX3 non-inv(16) cases. (**C** and **D**) Trend toward longer overall and relapse-free survival in unmethylated RUNX3 inv(16) AML cases but not statistically significant.

Table I

Sequences of primers used in this study.

Gene	Oligos	Sequence to Analyze
RUNX1	F: TGAATGAGAGTGTTTGGAAATGAA	GYGGTYGGYGGTTAATTATAGGTTTTT
Size: 123bp	R: Bio-AACTAATACCCAAAAAACCTATAATTAACC	
	P: TTTTTTAGTTAGTTTGTATAGGG	
RUNX2	F: GGGGTTGGATTGTTGAATTTATAT	GGGGYGTTAGYGTATTTAGGA
Size: 132 bp	R: Bio-CCTAAAAAAACCAAAAACTTAAATTTACA	
	P: ATAGGATTTTAATTGGGAG	
RUNX3	F: Bio-TTGTTTAGGGTTTGTGATTTTTGTAATT	ACRACRTCACCAA
Size: 113 bp	R: ACCAAACCAAAAAAATAAATAACCTCTTT	
	P: TTAAAACCACTCCCAAT	

Table II

Clinicopathological features of methylated and unmethylated *RUNX3* in AML patients.

Parameter	inv(16) Methylated (n= 13)		inv(16) Unmethylated (n= 3)		Non-inv(16) Methylated (n= 16)		Non-inv(16) Unmethylated (n= 32)		Mann-Whitney U-Test	
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	**inv(16): U vs M**	**non-Inv(16): U vs M**
Age, years	49.77	4.21	24.33	2.40	67.38	2.21	65.25	2.80	**0.015**	0.844
WBC, K/μL	35.73	8.70	41.03	25.15	11.97	6.00	20.39	4.73	0.893	0.106
Plt, K/μL	62.08	14.88	63.33	27.95	65.87	14.00	87.13	13.50	0.893	0.361
Hgb, g/dL	9.04	0.60	7.50	1.18	8.31	0.51	8.80	0.33	0.282	0.330
Blast, %	46.08	7.67	66.33	19.74	23.27	7.61	30.06	5.83	0.252	0.328
Mono, %	25.69	5.18	18.00	18.00	9.53	3.17	9.35	2.17	0.346	0.981
Neut, %	8.15	1.99	2.00	1.00	23.47	4.71	26.97	4.14	0.175	0.647
Promyelo, %	0.54	0.29	0.33	0.33	0.00	0.00	0.06	0.04	1.000	0.354
Alb, g/dL	3.25	0.19	3.20	0.46	3.59	0.19	3.39	0.12	0.946	0.359
LDH, IU/L	1788.85	469.96	1273.67	386.55	1080.13	284.84	1819.03	381.26	0.893	0.058
Bili, mg/dL	0.59	0.08	0.83	0.34	0.44	0.05	0.60	0.07	0.730	0.246
Creat, mg/dL	0.92	0.07	1.00	0.06	1.03	0.07	1.08	0.06	0.492	0.869
UA, mg/dL	4.48	0.38	4.80	0.60	4.67	0.45	4.87	0.42	0.563	0.879
Fibr, mg/dL	511.73	36.85	288.33	10.73	564.70	44.84	506.89	25.87	**0.013**	0.224
BM Bl, %	55.69	5.78	67.33	8.19	49.53	6.48	52.00	4.74	0.312	0.935
Bm Prog, %	1.92	0.54	0.67	0.33	0.67	0.23	0.84	0.20	0.405	0.796
BM Mon, %	6.77	1.95	4.00	2.52	2.47	1.06	3.65	1.25	0.685	0.971
BM Gran, %	4.54	1.57	4.67	0.88	7.27	2.39	8.26	2.09	0.408	0.860

WBC=white blood cell count. Plt=platelet count. Hgb=serum hemoglobin. Blast=bone marrow blasts. Mono=mononuclear cell count. Neut=neutrophil count. Promyelo=promyelocyte count. Alb=serum albumin. LDH=lactate dehydrogenase. Bili=serum bilirubin. Creat=serum creatinine. UA=serum uric acid. Fibr=fibroblast cultures. BM Bl=bone marrow blasts. Bm Prog=bone marrow progenitors. BM Mon=bone marrow monocytes. BM Gran=bone marrow granulocytes. U: unmethylated group. M: methylated group. P values below 0.05 are marked in bold.