Low level CpG island promoter methylation predicts a poor outcome in adult T-cell acute lymphoblastic leukemia

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Supplementary methods

Methylation classifier

In order to develop a classifier of gene promoter methylation markers which is predictive of the hyper-methylated group, we computed a correlation coefficient for each of the 17,970 promoter regions using “neighborhood analysis”. Regions with an absolute coefficient greater than 3.5 were retained for subsequent training and testing. Eleven gene promoters were selected and used their degree of correlation (Wi) as weights between the two distinct classes (1). We randomly created 10 subsets of 12 samples issued from the original training data set (25 samples). The prediction score for each patient in the 10 randomly training sets was then computed according to the established weights, following the procedure described in (2):

1) Center the methylation level of each feature to the corresponding feature mean of the subset;
2) Multiply the centered methylation level by the corresponding feature weight;
3) Sum the weighted methylation levels as single predictive score for each patient.
4) Compute the best Threshold Score of Prediction (TSP) (~1.48) with these random subsets.

Next, we made a prediction model to challenge the training and test data sets. We computed the prediction score as described above and correlated them with the TPS to assign each sample to either the hypermethylated (TSP > 1.48) or the hypomethylated (TSP < 1.48) group.

Statistical analysis

Comparison of continuous and categorical variables between subgroups was performed by Mann-Whitney test and Fisher’s exact test, respectively. The Overall Survival (OS) was calculated from the date of prephase initiation to death date censoring patients alive at last follow-up. The cumulative incidence of relapse (CIR) corresponds to the time from complete remission date to relapse date censoring patients alive without relapse at last follow-up date and considering death in complete remission (CR) as a competitive event. Univariate and multivariate analyses were performed with a Cox model for OS and a Fine-Gray model for CIR. Proportional-hazards assumption was checked before conducting multivariate analyses.

Variables associated with OS or CIR in univariate analyses with a $p<0.1$ were considered to be included as covariates in multivariable models. Statistical analyses were performed with the STATA software (STATA 12.0 Corporation, College Station, TX). All tests were two-sided with a significance level of 0.05.

Supplementary references

1. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science (New York, NY. 1999 Oct 15;286(5439):531-7.
2. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, et al. DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. Cancer cell. 2010 Jan 19;17(1):13-27.

Supplementary figures.

Figure S1: Global promoter methylation in T-ALL and normal thymic samples. The methylation score is calculated from the median enrichment ratio of overlapping promoter probes.

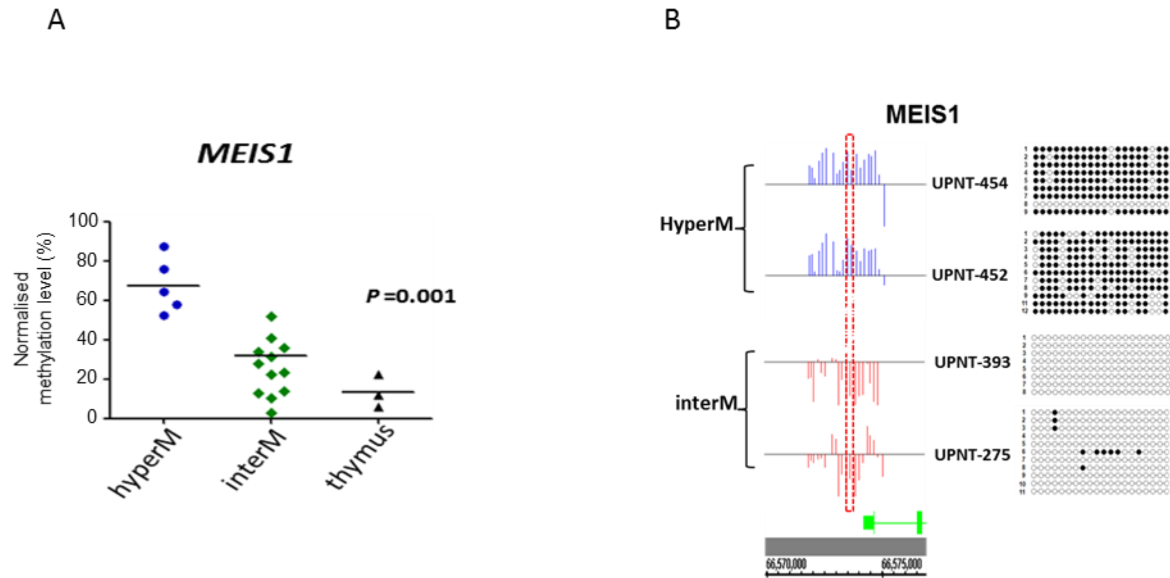

Figure S2: Validation of the methylation levels. A) Methylation of the *MEIS1* promoter was assessed by qPCR analyses of MeDIP on a subset of hyperM and interM T-ALL samples, along with human thymus. The methylation levels were normalized with respect to an *in vitro* methylated genomic DNA. Statistical significance between the hyperM and interM subgroups was assessed by unpaired T test. B) Methylation of the highlighted region (dashed box) of the *MEIS1* promoter was assessed by bisulfite sequencing of two hyperM and two interM T-ALL samples. Each line represents a sequenced clone, where black and white circles indicate methylated and unmethylated CpG.

Supplementary Table S1. MeDIP Methylation scores per promoter in T-ALL samples

Supplementary Table S2. MeDIP Methylation scores per promoter in T-LBL samples

Supplementary Table S3. Differentially methylated promoters in T-ALL and T-LBL

Gene	LHS	RHS
BMP4	CCTCTCGGTTTTAGAACCGCGCTCTCC	CGCCCCAGGAGATTCCTTGGGGCCGAG
HOXB7	TTCCTTCAACATGCACTGCGCGCCCTTTGAG	CAGAACCTCTCCGGGGTGTGTCCCGGCGACT
KCNA1	TATTCCAGGCGCTTTCTCAGGTTTCTGCTGATCTT	GCAGCGCCCAGAAATGGACCGAGCGGACCCGCCGCC
LHX1	TCCTTCCCCCGCGCCGGCGCCGCGCCTCCGGTCTCCC	CGCCCCCATCAGGAAACCGCCCGAATCAACTTTGCAAG
MEIS1	GAGGAAAGTCATGAAGTCTATGCGCGGAGCCCTGTGCAA	AATAACTCCCGCTGCTGCCTGCCCGGCGTTGATTCCCAAT
PROX1	GTCCTGGAAGAGCTAGTGTGAGCCGGGCGCCGCTCGCGCCG	TCTCCCGCTTTGCATAGTGCCCGCAGATGGCTCGCTCCGGCC
PSD3	GCCCCGGAGCGCCCGGCGGCGGTTTCGGCGCGCGGCCGGGCTGGC	GATGGAAGATGGAAGGAAGGAGCGCAGCGGTGAGCTCCGGGGCCGG
RUNX2	GAGTAGTATCCCCTGAACTCCATCCTTACCCCTCGAGAGCGCACACC	TGGCTACCCCGCACCCCCACCTCTGCTCCCGCGGTCTGGCAGACCCTC
SEMA6A	CTGTGCCTGCCATTCTTCATGTGATCATAACAATAGCGCCTTGGAAGTG	GTTGCGATTTTCTTCTTCCATAAACCTTTTGGGTTCTCACTGGAATTGTA

Supplementary Table S4. Methylation-specific multiplex ligation-dependent probes on the 9 promoter regions forming the methylation classifier. LHS: left hybridizing sequence, RHS: right hybridizing sequence.

	Low methylation N=42 (Q1)	Int/High methylation N=126 (Q2-Q4)	p-value†
oncogenetic classifier			
NOTCH1/FBXW7 mutation	18/42 (43%)	99/126 (79%)	**<0.0001**
RAS mutation	3/42 (7%)	13/126 (10%)	0.76
PTEN alteration	14/42 (33%)	8/125 (6%)	**<0.0001**

Supplementary Table S5. The incidence of NOTCH-activating mutations (NOTCH/FBXW7), RAS mutations and PTEN alterations (mutations or deletions) found in the low methylated subgroup (CIMP-negative) compared to the Intermediate/ High methylated subgroup.