DOI: 10.3324/haematol.2010.028605

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Table 1. PCR primers and probes for PcG protein genes.

Gene name (Accession no.)		Primer and probe sequence	Product size (bp)
BMI1 (NM_005180)	F	5'-GCCTACATTTATTCCTGGAGAAG-3'	135
	R	5'-CCCAGAGTCACTTTCCAGTT-3'	
	P	5'-FAM-TTGTCAGTCCATCTCTCTGGTGACTGATCT-TAMRA-3'	
YY1 (NM_003403)	F	5'-CAACAAGAAGTGGGAGCAG-3'	143
	R	5'-GAGGTGAGTTCTCTCCAATGAT-3'	
	P	5'-FAM-CTCGGTCACCATGTGGTCCTCAGATGA-TAMRA-3'	
RYBP (NM_012234)	F	5'-CTGACATTCTGAAAGATCCTCC-3'	143
	R	5'-AGTTACTGCCAACTGCTGTG-3'	

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	P	5'-FAM-TGCAAATGCTACAACAAAGACCAGCGA-TAMRA-3'	
RBBP4 (NM_05610)	F	5'-ATGCCCCAGAACCCTTGT-3'	132
	R	5'-ATGTCCACGGAGACGCAA-3'	
	P	5'-FAM-CTCCTTCCAGTGATGTTCTTGTCTTTGACT-TAMRA-3'	
EED (NM_152991)	F	5'-GAATATCCAGACGGACACTC-3'	126
	R	5'-AGAGAATGATCCATACCACAG-3'	
	P	5'-FAM-ATAATCAGCACTTAGAACTTCATCTCTGTGCC-TAMRA-3'	
EZH2 (NM_152998)	F	5'-GATGTGGATACTCCTCCAAG-3'	149
	R	5'-GAACTGTCACAAGGCTGC-3'	
	P	5'-FAM-ACGGCTCCTCTAACCATGTTTACAACTATCA-TAMRA-3'	
PBGD (NM_000190)	F	5'-AACCAGCTCCCTGCGAAGA-3'	134
	R	5'-CCAGGATGATGGCACTGAACT-3'	
	P	5'-FAM-ACTCCTGAACTCCAGATGCGGGAACT-TAMRA-3'	

F: forward primer, R: reverse primer, P: TaqMan probe

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Figure legends

Figure 1. Microarray analysis of gene expression in primary ATL cells. (A-D) Expression levels of PcG protein genes were compared among normal CD4+ T cells (Control), chronic ATL cells (Chronic), and acute ATL cells (Acute), and results of *EZH2* (A), *RYBP* (B), *BMI1* (C), and *CBX7* (D) are demonstrated in box plots. ATL cells showed significantly higher levels of *EZH2* and *RYBP* transcripts than normal CD4+ T-cells (Mann-Whitney's U test), with a higher expression in the acute type than in the chronic type (Mann-Whitney's U test) (A, B). In contrast, there was no statistical difference in the level for *BMI1* or *CBX7* among these groups (C, D). (E-H) Overall survival curves for ATL patients separated into two groups consisting of those with high expression (H, n=20) and low expression (L, n=20) for *EZH2* (E), *RYBP* (F), *BMI1* (G), or *CBX7* (H) are shown. Patients with high *EZH2* or high *RYBP* expression showed significantly shorter survival than those in corresponding low expression groups (Log-rank test) (E, F). There was no difference in survival for different *BMI1* or *CBX7* expressions (G, H). H: high expression group (bold line), L: low expression group (thin dotted line). $*p<0.05$, $**p<0.01$

Figure 2. Quantitative real-time RT-PCR for PcG genes. (A-F, a-f) Expressions of PcG protein genes *EZH2* (A, a), *RYBP* (B, b), *RBBP4* (C, c), *BMI1* (D, d), *YY1* (E, e), and *EED* (F, f) were compared among healthy adults (Control), HTLV-1 carriers (Carrier), ATL patients (Primary ATL), ATL cell lines, and non-ATL T-cell lines. Capital letters (A-F) indicate absolute copy number per 25 ng of total RNA, and small letters (a-f) indicate normalized expression. ATL cells showed significantly higher levels of *EZH2* and *RYBP* transcripts than

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the cells from healthy adults and HTLV-1 carriers, in terms of both absolute copy number and normalized expression (A, a, B, b, Mann-Whitney's U test). *RBBP4* transcript was significantly increased in ATL cells only in terms of normalized expression (C, c, Mann-Whitney's U test). There was no difference in *BMI1*, *YY1*, and *EED* expression levels among these groups (D, d, E, e, F, f). $**p<0.01$

Figure 3. EZH2 protein expression and histone methylation. (A) Western blot analysis for EZH2 protein was performed on primary ATL cells, cells from healthy adults, and ATL cell lines. Primary ATL cells showed a clear 98-kDa band for EZH2 with the absence or presence of faint bands for phosphorylated EZH2 (p-EZH2). Cells from healthy adults hardly showed these bands. ATL cell lines ST1, SO4, and KK1 showed intense bands for both EZH2 and p-EZH2, but LM-Y1 and KOB cells showed intense bands for EZH2 with the absence of a band for p-EZH2. (B) Western blot analysis for histone methylation status was performed. Only primary ATL cells and LM-Y1 and KOB cell lines showed a clear band for H3K27me3, but others hardly showed the band. Bands for H3K27me2, H3K27me1, and histone H3 were observed in almost all samples examined.

Figure 4. Immunostaining for EZH2 and H3K27me3 in lymph nodes. Lymph nodes from patients with lymphoma-type ATL and follicular lymphoma (FL) were stained for EZH2 and H3K27me3. Representative results of 3 ATL lymph nodes and 1 FL lymph node are shown. ATL lymph nodes were all strongly positive for both EZH2 and H3K27me3 without exception in their cell

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nuclear staining (brown color). In contrast, FL lymph nodes were sparsely positive for EZH2 and mostly negative for H3K27me3. HE: hematoxylin-eosin stain. EZH2 and H3K27me3: immunostaining, Nikon Eclipse 80i, magnification ×200.

Figure 5. Quantitative real-time RT-PCR for miRNAs. (A-C) Expressions of miR-101 (A), miR-26a (B), and miR-128a (C) were compared between ATL patients and HTLV-1 carriers. Primary ATL cells showed significantly lower levels of miR-101 and miR-128a (Mann-Whitney's U test) compared with the cells from HTLV-1 carriers (A, C). There was no significant difference in miR-26a expression between the two groups (B). (D, E, F) Correlation between miRNA and *EZH2* or *BMI1* expression was examined. There were significant inverse correlations between normalized *EZH2* expression and miR-101 expression (D) or between normalized *EZH2* expression and miR-128a expression (E) (Spearman's correlation coefficient). In contrast, there was no correlation between normalized *BMI1* expression and miR-128a expression (F). $*p<0.05$, $**p<0.01$

Figure 6. Sensitivities of cell lines to DZNep and PS (LBH589). (A) Sensitivities of cell lines to DZNep were examined after 72 hours of culture. DZNep suppressed the proliferation of all cell lines examined at concentrations above 0.5 μM but showed no effect on normal $CD4^+$ T cells (control 1-4, dotted lines). (B, C) Effects of DZNep on *EZH2* transcript (B) or EZH2 protein expression (C) were examined in ATL and HTLV-1-infected cell lines. DZNep was added at final concentrations of 0.5 and 5 nM. DZNep decreased *EZH2*

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transcript in ST1, SO4, and KK1 but increased it in KOB (B), which results were confirmed at protein level (C). (D, E) Effects of PS (LBH589) on *EZH2* transcript (D) or EZH2 protein expression (E) were examined. PS (LBH589) was added at final concentrations of 50 nM and 100 nM for (D) and 20 nM and 100 nM for (E). One hundred nM of PS (LBH589) decreased the expression of EZH2 at both transcript (D) and protein levels (E) after 24 hours of culture. (F) Effects of combined treatment with DZNep and PS (LBH589) on LM-Y1 and KOB cells were analyzed. Cells were treated with DZNep (0.3-5.0 μM) and PS (LBH589) (3-50 nM) for 48 hours. After evaluation of cell proliferation status by a MTS assay (upper panel), the combination index (CI) for each drug combination was obtained using commercially available software Calcusyn (lower panel). CI < 1 indicates synergism.

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Figure 1

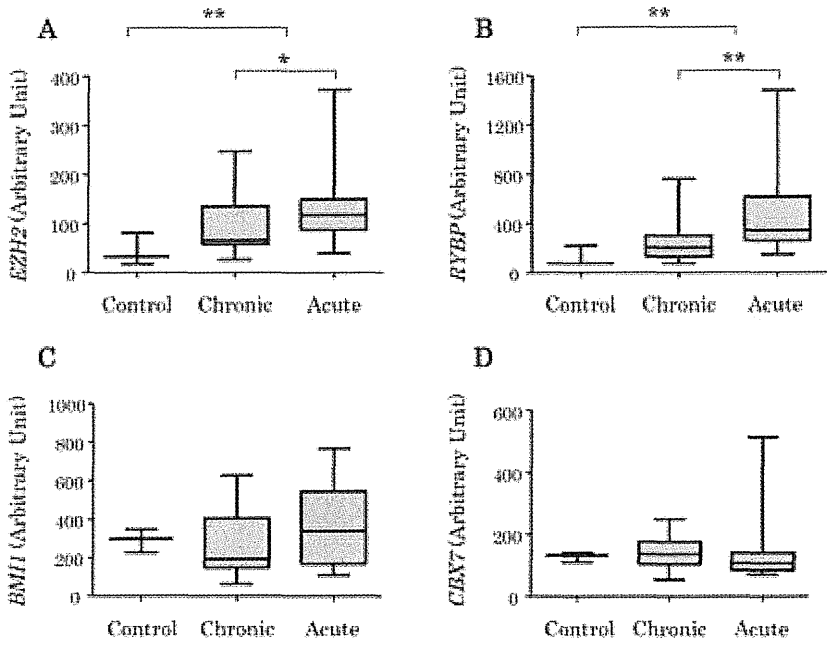

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Figure 1

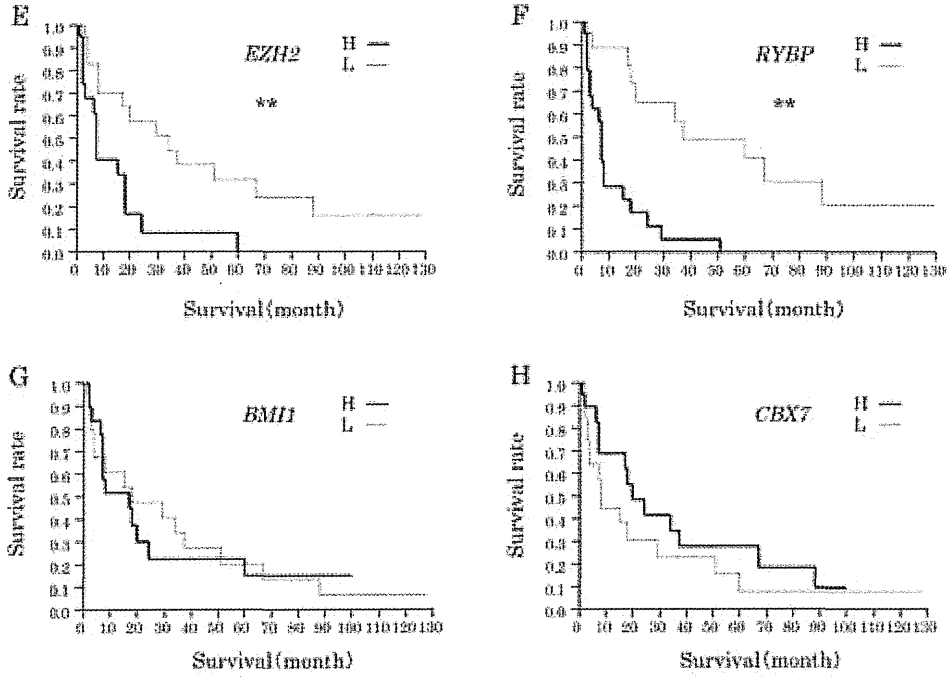

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Figure 2

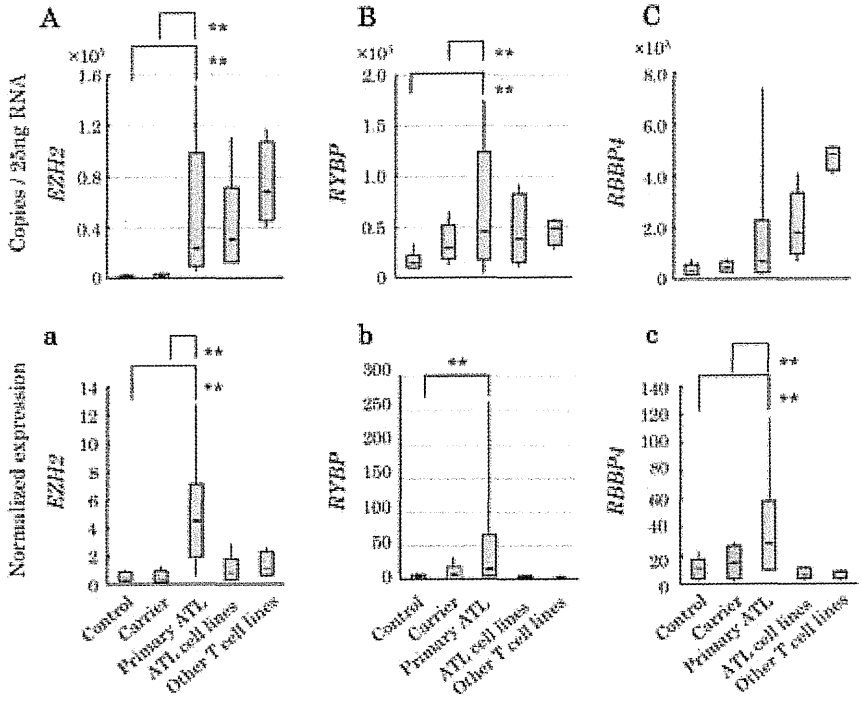

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Figure 2

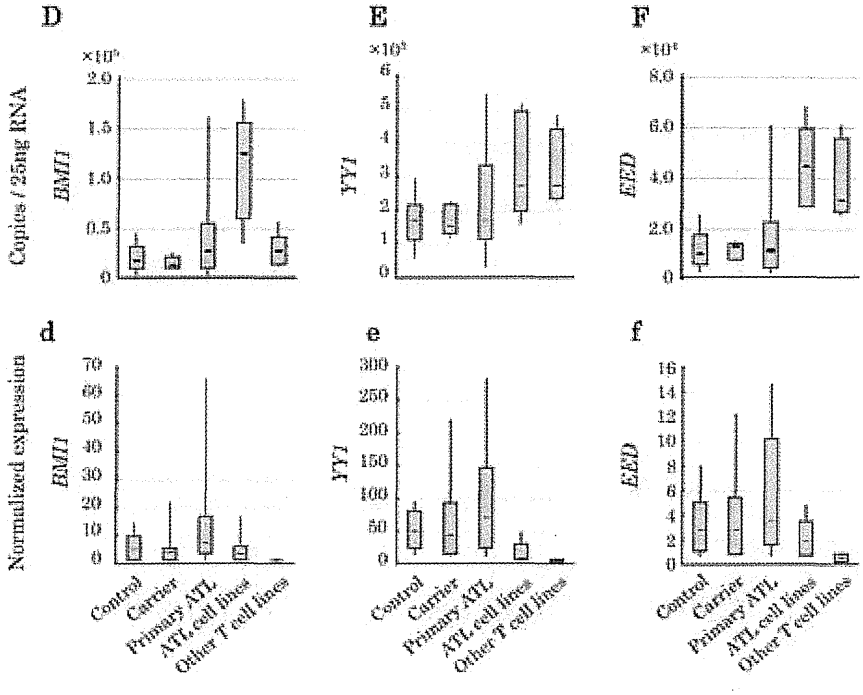

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Figure 3

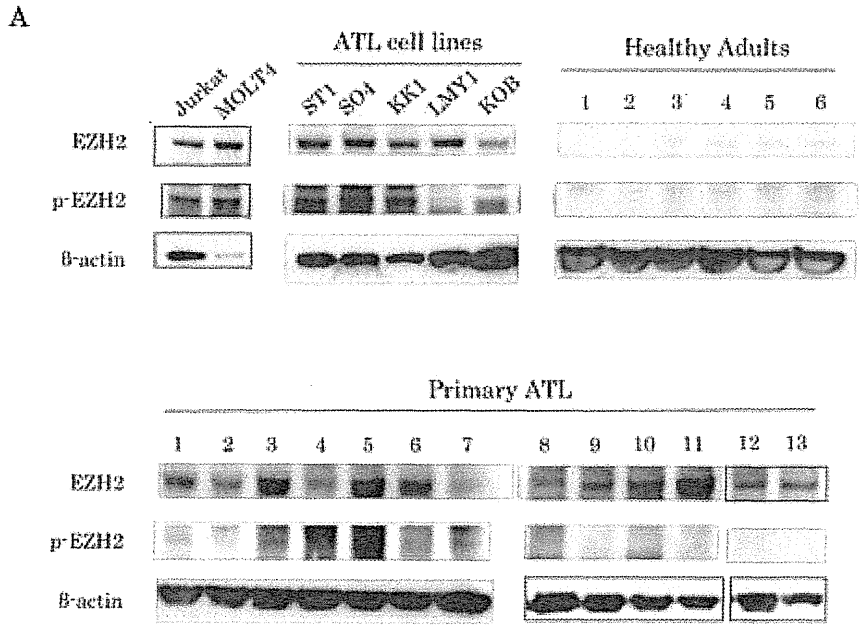

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Figure 3

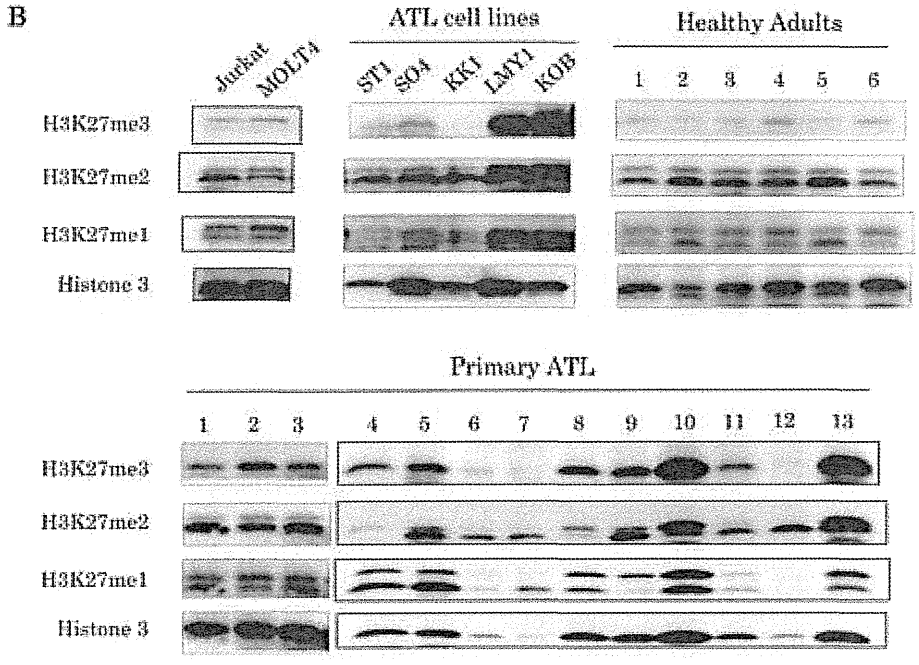

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Figure 4