SPANDIDOS PUBLICATIONS

Expression of Dlk1 gene in myelodysplastic syndrome determined by microarray, and its effects on leukemia cells

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Abstract. Delta-like-1 (Dlk1, also Pref-1), a transmembrane and secreted protein, is a member of the epidermal growth factor-like family, homologous to Notch/Delta/Serrate. We found that the expression of Dlk1 was up-regulated in $CD34^+$ cells from patients with myelodysplastic syndrome (MDS) by analyzing the gene expression profiles determined by microarray. The expression levels of Dlk1 mRNA frequently observed higher in the bone marrow mononuclear cells of MDS patients was confirmed by real-time RT-PCR. Forced expression of Dlk1 in transfected K562 cells enhanced proliferation, affected apoptosis induced by As_2O_3, and also influenced cell cycle induced by 12-O-tetra decanoylphorbol-acetate (TPA). By using the same experimental system we found that forced expression of Dlk1 increased the mRNA levels of HES1. It also inhibited p38 phosphorylation in transfected K562 cells treated with TPA. These results warrant further investigation of the role of Dlk1 in abnormal hematopoiesis in MDS.

Introduction

Myelodysplastic syndrome (MDS) is a clonal hematologic disorder characterized clinically and morphologically by ineffective hematopoiesis (1). Its features include refractory cytopenia, dysplastic cellular morphology, and a propensity toward malignant transformation (2,3).

The natural history of this syndrome ranges from a chronic course that may span years to a rapid course of leukemic progression. Prognosis of this disease is most closely related to the percentage of marrow myeloblasts and also to the presence and features of specific cytogenetic abnormalities (3,4). In general, myelodysplastic syndrome is a preleukemic condition in which the neoplastic clone has been established, but not all MDS cases terminate in acute myeloid leukemia (AML) (1).

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The molecular pathogenesis of MDS remains poorly understood. A proposal for the multistep pathogenesis of MDS suggests that the initial damage in hematopoietic stem cells can affect the expression of cell cycle-related genes, including checkpoint and mismatch repair genes, transcription factors, and tumor suppressor genes. Recent studies indicate that an increased expression of the Delta-like-1 (Dlk1) gene is frequently observed in the $CD34^+$ cells from MDS patients (5-8).

The Dlk1 gene presents in animals from birds to mammals and is known as an imprinted gene (9). The protein is expressed in a variety of fetal and selected adult tissues and is thought to participate in embryonic growth, hematopoiesis (10) and wound healing (11). Moore *et al* (12) and Ohno *et al* (13) showed that Dlk1 was preferentially expressed in fetal stromal cell lines which supported hematopoietic stem cell growth, but not in nonsupportive tissues. They directly demonstrated that exogenous Dlk1 was a positive regulator of murine stem cell growth. Dlk1 also regulates the growth of pre-B cells and thymocytes (14,15). However, these studies focused on the effects of exogenous Dlk1 on hematopoietic cells. The functional consequences of aberrant, intrinsic Dlk1 overexpression in human hematopoietic cells are not known (16,17).

Materials and methods

Patients. $CD34^+$ cell samples isolated for microarray analysis from eight patients [comprising two cases of RA, one RAS, two RAEB, two RAEBt, and one case of chronic aplastic anemia (CAA)] (Table I), and one healthy individual, were subjected to microarray hybridization for gene expression profiling. For real-time PCR, bone marrow mononuclear cells were collected from another 19 MDS samples (seven RA, five RAS, and seven RAEB and RAEBt samples), six AML samples and 10 other samples (one sample of pure megakaryotic anemia, four IDA, one hypercellular anemia, and one ITP sample, and samples from three healthy individuals). Patients were classified as having refractory anemia (RA) or refractory anemia with excess blasts (RAEB) according to the FAB criteria.

Sample preparation. Heparinized bone marrow samples were obtained by aspiration from the posterior iliac crest after informed consent. Fresh bone marrow was processed immediately after aspiration to select the $CD34^+$ cells or mononuclear cells (MNC) within 4 h. $CD34^+$ cells were purified

according to established protocol (5-8). Mononuclear cells were separated by density gradient Ficoll-Hypaque centrifugation. Total RNA was extracted using TRIzol (Invitrogen, Shanghai, P.R. China) according to the manufacturer's protocol. Due to the relative hypocellularity in the bone marrow of MDS patients, especially in MDS at RA stage, only 0.5-1.0x10^5 mononuclear cells could be obtained from a single aspiration and the number of $CD34^+$ cells was even lower at 0.5-1.0x10^4.

Oligonucleotide microarray and data analysis. Nine pieces of Human Genome U133 Plus 2.0 Array were used. Eight chips were hybridized with amplified products from eight patients and one from the healthy control. A detailed protocol for the sample preparation and microarray processing is available from Affymetrix (Santa Clara, CA, USA). Due to the small number of cells from which the RNAs could be extracted, two rounds of *in vitro* transcript amplification were needed before hybridization on the microarray. The amplified products were checked for no marked distortions in RNA species.

GeneChip image analysis using Microarray Analysis Suites version 5.0. Data analysis was performed with GeneSpring software version 6.0. Samples were normalized for expression levels in each chip. Differences in expression level using the filter on fold changes and statistical analysis [analysis of variance (ANOVA)] were then calculated for all individual genes in the test samples from the MDS patients, based on comparison with healthy controls. Fold changes for the log ratios were shown. A signal log2 ratio ≥1 was considered 'up', whereas a signal log ratio ≤-1 was considered 'down'. Differential gene expression patterns were calculated using a hierarchical-clustering algorithm as a gene tree or a condition tree to display the gene expression profile (5-8). Genes of interest were selected for bioinformatics study.

Quantitative real-time RT-PCR. Quantification of RNA in bone marrow mononuclear cells by real-time PCR was performed as described previously. Briefly, 500 ng total RNA was processed directly to cDNA by reverse transcription. PCR primers and TaqMan probes were designed using Primer Express 2.0 software with published sequence data from the NCBI database. Amplification reactions contained 2 μl cDNA, 1 μl 20X buffer, 2 μl $MgCl_2$ 4 μl 5X Q-Solution, 0.8 mM dNTPs, 2 μM primers, 1 μM probes, 1 U HotStar (Qiagen, Germany). In a final volume of 20 μl, the thermal cycling conditions were as follows: 15 min at 95˚C, followed by 40 cycles at 95˚C for 30 sec, 50˚C for 30 sec, and 72˚C for 30 sec. ß-actin was used as an active and endogenous reference to correct for differences in the amount of total RNA added to a reaction and to compensate for different levels of inhibition during reverse transcription of RNA during PCR.

Cell transfection and Western blot analysis. K562 leukemia cells were cultured in RPMI-1640 containing 10% FCS. The expression vector pcDNA-Dlk1 was constructed by placing full-length human Dlk1 cDNA into the pcDNA3.0 vector (Invitrogen). The constructs were transfected into K562 cells by lipofectamine and stable transfectants were selected for their G418 resistance (800 mg/ml). Empty vector was also transfected at the same time.

The Dlk1 overexpressing clones were detected by Western blot analysis. Anti-Dlk1 polyclonal antibody (C-19) (Santa Cruz, CA, USA) and anti-actin antibody (Research Diagnostic, Flanders, USA) were applied. Anti-phosphorylated p38 and p38 antibodies were also used (Cell Signaling, USA).

Cell proliferation in liquid culture assays. Viable cells were counted using Cell Counting Kit-8 (CCK-8) beginning in 96-well plates at 2-10^3 cells/well; 10 μl CCK-8 solution was added to each well, after 2 h of incubation, the optical absorbance at 450 nm was measured. Each experiment was performed three times.

Flow cytometric analysis for cell cycles. After culture without FCS for 24 h, 5 nM 12-O-tetra decanoylphorbol-acetate (TPA) (Sigma, St Louis, MO, USA) and 10% FCS were added into the cell culture followed by continuous culture for 18 h, after which cells were harvested and washed three times by 5 ml PBS/0.1% FCS wash buffer, centrifuged and aspirated. The cells were then re-suspended with 20 μg/ml PI, 500 μg/ml RNase and 0.03% NP-40 in wash buffer, and analyzed by flow cytometry (FACScan) (Becton Dickinson, Heidelberg, Germany).

Apoptosis assessment by annexin-V staining. After As_2O_3 treatment for 3 h, cells were washed in PBS and re-suspended in 100 μl staining solution (containing annexin-V fluorescein and PI in a HEPES buffer, Annexin-V-FLUOS Staining Kit, Boehringer-Mannheim). Following incubation at room temperature for 15 min, cells were analyzed by flow cytometry. Annexin-V binds to cells which express phosphotidylserine on the outer layer of the cell membrane, and PI stains the cellular DNA of those that have a compromised cell membrane. This allows for the discrimination of live cells (unstained with either fluorochrome) from apoptotic cells (stained only with annexin-V) and necrotic cells (stained with both annexin-V and PI) (18).

Results

Gene transcription profile of $CD34^+$ cells. The 18,404 signal dots on the chips were filtered for the final clustering and bioinformatics analysis (Fig. 1a). About 164 genes or ESTs were down-regulated in the $CD34^+$ cells of all 7 MDS samples in contrast to healthy controls (Fig. 1b), while 46 genes or ESTs were up-regulated in all 7 MDS samples compared with controls (Fig. 1c). Twenty-three genes or ESTs were identified for their differential expression between MDS-RA or -RAS and CAA patients. Fifteen genes were highly expressed in MDS-RA and MDS-RAS samples, while they were down-regulated in CAA; eight genes were down-regulated in the MDS-RA and MDS-RAS cases, while they were highly expressed in CAA samples. (Fig. 1d, Table II). These genes are likely to be involved in the regulation of hematopoietic proliferation and differentiation.

Expression of Dlk1 was frequently up-regulated in MNCs of MDS. Among the genes or ESTs which were identified for

Characteristics of patients from whom samples were obtained for microarray.

Patient	Age (years)	Gender	FAB	Karyotype	IPSS
RAEBt1	34	M	RAEBt	46,XY,tandem duplication(1)(q12q24)	HIGH
RAEBt2	70	F	RAEBt	47,XX,+8 [4]/46,XX [9]	HIGH
RAEB1	43	M	RAEB	43,XY,5q-,6p+,-7,-der(12)t(12;18)(q11;q23), dic(15;21),der(18)t(12;18)[8]/46,XY[1]	INT-2
RAEB2	40	F	RAEB	46,XX,der(6)[3]/47,idem,+8[7]	INT-2
RA1	53	F	RA	RA/47,XX,+8,9q-[4]/48,idem,+der(1)[7]/48, +der(1),9q-,+9q-,+mar[CP2]/46,XX[2]	INT-2
RA2	40	M	RA	44,XY,del(5)(q12q31),-7,-18[9]/46,XY[2]	INT-2
RAS	51	M	RAS	45,XY,-5,-6,+mar[7]	INT-1
CAA	30	F	CAA	46,XX	

M, male; F, female.

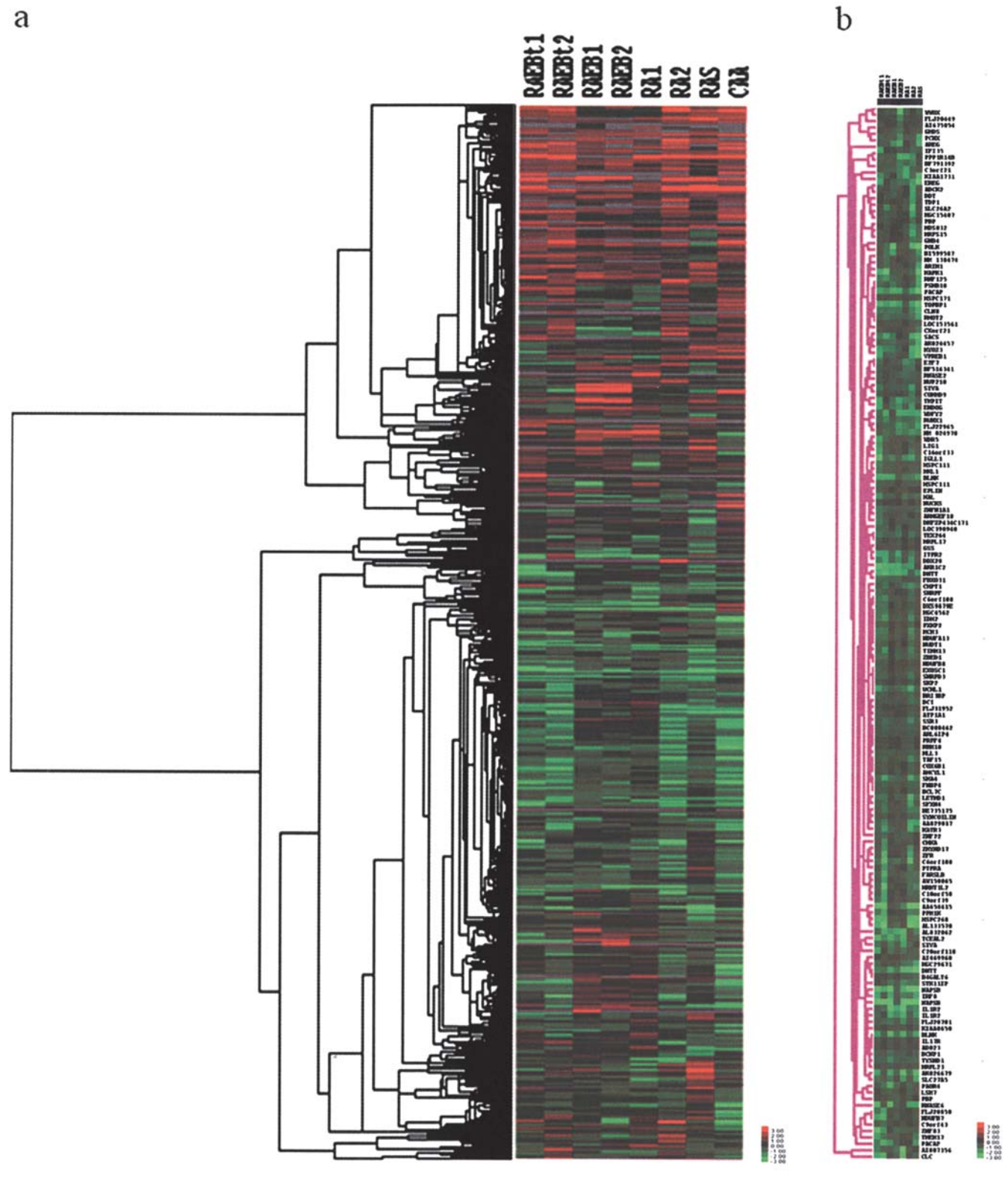
Table II. Partial list of genes with differentially expressed patterns in RA or RAS groups and in CAA.[a]

UNIQID	RA1	RA2	RAS	CAA	UniGene ID	Chromosomal location
ZFHX1B	-1.5	-1.8	-1.5	1.0	Hs.34871	chr2q22
LOC166994	-1.4	-1.8	-4.5	1.3		chr6p22.1
LOC440253	-1.1	-1.3	-1.4	1.3		chr15q13.1
LOC285527	-1.3	-1.1	-2.4	1.4		chr4p12
TRPM7	-1.8	-2.4	-1.5	1.6	Hs.512894	chr15q21
AQP9	-3.7	-1.4	-1.5	2.0	Hs.104624	chr15q22.1-q22.2
S100A8	-1.0	-3.1	-1.3	1.6	Hs.416073	chr1q21
LOC91353	-1.9	-1.2	-1.5	2.1	Hs.567636	chr22q11.23
AK026679	-5.1	-2.8	-4.7	1.3	Hs.677385	hr21
PACAP	-2.3	-3.5	-5.3	2.2	Hs.409563	chr5q23-q31
MIRN21	-1.1	-1.5	-2.5	1.2		chr17
AU155234	-1.5	-2.3	-1.2	1.2		
BE552357	-2.5	-1.6	-3.4	1.1		
BE551146	-1.4	-2.1	-2.5	1.0	Hs.667826	chr20
SPPL3	-2.3	-1.6	-3.9	2.2	Hs.507087	chr2q24.31
YWHAH	1.2	1.1	1.2	-2.7	Hs.226755	chr22q12.3
PSMF1	1.1	1.6	1.4	-1.5	Hs.471917	chr20p13
PTGS1	1.0	1.6	1.2	-2.1	Hs.201978	chr9q32-q33.3
CX3CR1	1.1	3.9	1.0	-2.4	Hs.78913	chr3p21.3
DLK1	3.7	1.2	1.1	-1.6	Hs.533717	chr14q32
ARFGEF1	1.4	1.2	1.0	-2.3	Hs.411848	chr8q13
SNRPN	1.8	1.0	1.7	-2.6	Hs.525700	chr15q11.2
ATF6	1.0	1.5	1.4	-1.9	Hs.492740	chr1q22-q23

[a]Fold change from log2 ratios is shown.

their differential expression between MDS-RA and CAA patients, Dlk1 was always up-regulated in MDS compared with healthy controls in all seven gene chips, while in CAA Dlk1 was down-regulated in $CD34^+$ cells.

The median levels of Dlk1 transcript were 2.55 (range, 0.00-23.7) in 12 patients with MDS-RA and -RAS, 8.24 (range, 2.01-18.44) in 7 MDS-RAEB and -RAEBt, 1.88 (range, 0.12-5.13) in 6 AML patients, and 0.37 (range, 0.00-1.79) in the 10 other individuals, respectively. The abundance of Dlk1 mRNA relative to that of ß-actin mRNA in the cells from most MDS patients was markedly greater than that in the MNCs from the others ($P<0.05$). The ratio of Dlk/ß-actin



in MDS-RAEB and RAEBt was markedly higher than that in AML ($P<0.05$). Among the MDS subtypes, there were no significant differences in the Dlk1 transcript levels (Fig. 1d).

Dlk1 caused cellular proliferation of K562 cells. To examine the effect of Dlk1 on cell proliferation, we generated K562 cells stably overexpressing Dlk1. Three stable clones expressing Dlk1 and control clones (transfer vector) were isolated (Fig. 2a). The Dlk1 expressing clones grew faster than controls (Fig. 2b). To elucidate the mechanisms of the action of Dlk1 on cell growth, we analyzed the levels of cell proliferation gene in these cell lines. Using RT-PCR, the HES1 were found up-regulated in these stable clones which were overexpressing Dlk1 (Fig. 2c), while the transcriptional expression of Notch1, Notch2, NF-κB, and $p21^{WAF1}$ transcript variant 2 were no different (data not shown). In addition, no difference in the expression of ERK, JNK, and p38 at protein level was found by Western blot analysis (data not shown).

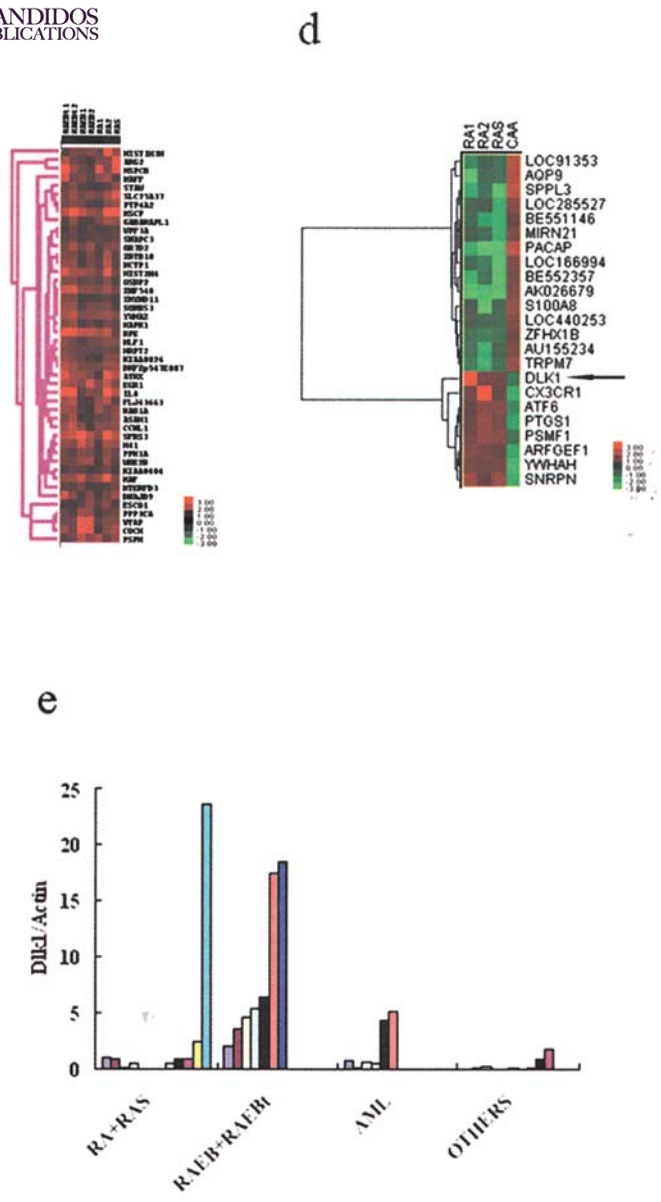


Figure 1. Dlk1 was frequently up-regulated in myelodysplastic syndrome (MDS). (a) Identification of genes expressed in CD34+ cells distinguishes between patients and healthy controls. Each row represents a separate patient sample, and each column represents a gene or EST on the microarray. The clustering is presented graphically as a colored image. Along the horizontal axis, the analyzed genes are arranged in order of the clustering algorithm. The genes with the most similar patterns of expression are placed adjacent to each other. Along the vertical axis, samples are arranged with the most similar patterns of expression across all genes placed adjacent to each other. The color and intensity of each cell in the image represents the expression level of each gene, with red representing an expression greater than the mean, and green representing an expression less than the mean, with a brighter color intensity representing a greater magnitude of deviation from the mean. (b) Hierarchial clustering of certain genes that were down-regulated in all MDS samples. (c) Hierarchial clustering of certain genes that were up-regulated in all MDS samples. (d) Hierarchial clustering of certain genes that had differentially expressed patterns in RA or RAS groups and in CAA. (e) Quantification of Dlk1 mRNA in the BMNC Bank samples from patients with MDS, AML, and others by real-time RT-PCR. Bone marrow mononuclear cells selected from 19 MDS samples (including 7 RA, 5 RAS, 7 RAEB and RAEBt) 6 AML samples and 10 other samples (including 1 PMA, 4 IDA, 1 hypercelluar anemia, 1 ITP, and 3 normal samples). Data were analyzed according to the comparative cycle threshold method and normalized by ß-actin in each sample. Dlk1 primers (forward, 5'-GTACTCGGGAAAGGACTGCC-3'; reversal, 5'-CTCGCAGAAATTGCCTGAGA-3'; and probe, 5'-FAM-AGGCACCCGTGGATGATGAG-TAMRA-3') were used.

Dlk1 affects apoptosis of K562 cells induced by As_2O_3. Both the Dlk1-transfected and control K562 cells were treated by 0.2 μM As_2O_3 for 3 h without FBS. The cells were stained by annexin-V and analysed by flow cytometry. Fourteen percent of the cells in control clone were positive for annexin-V staining while very few annexin-V positive stained cells were detected in the stable transfected K562 clones overexpressing Dlk1 (Fig. 3a).

Dlk1 influences cell cycle phase distribution upon TPA induction. The cell cycle phase distribution analysis by flow cytometry on the K562 cells treated by TPA showed a markedly higher number of control clone cells in G2 phase than stable transfectant clone cells expressing exogenous Dlk1 (Fig. 3b and c). To elucidate the relevance of the Dlk1 gene to the MAPK pathway, we analyzed the protein levels of both phosphorylated-p38 and p38 in cells exposed to TPA. In three stable K562 clones overexpressing Dlk1 the phosphorylated-p38 protein was markedly reduced compared to the control clones, while no difference was found in the p38 protein level between these two groups (Fig. 3d).

Discussion

Gene expression profiles in the hematopoietic cells of MDS patients have been studied using microarray, by our group and others during recent years in the hope of identifying candidate genes which may assist in furthering our knowledge of the mechanisms of the development and progression of MDS. Furthermore, such genes would probably provide biomarkers in the future, facilitating the diagnosis of MDS. Our previous study identified a handful of candidate genes which were differentially expressed in the bone marrow mononuclear cells (MNCs) of MDS patients in comparison with normal controls using a cDNA microarray designed by our group (19). The expression pattern of some of these genes, for example, the C/EBPξ gene, was confirmed by quantitative real-time RT-PCR in an expanded cohort of MDS patients (20). However, MNCs in MDS patients may not represent the dysplastic clone due to the extremely heterogeneous entity of the disease, and the gene expression pattern of MNCs may mask the real expression profile of MDS clone. The quality of the array also influences the reliability and reproducibility of the results. In the present study, we isolated CD34+ cells from the BM of MDS patients, extracted RNA, and applied high quality Affymetrix chips to improve our results. The results of our present study are somewhat discrepant from our previous results in terms of the expression pattern of some genes. We revealed a distinct gene expression profile in MDS patients in the present study. In addition, we identified certain genes whose expression pattern may distinguish low-risk MDS from CAA and MDS-RA/RAS from MDS-RAEB patients. Among these genes displaying different expression patterns between MDS and CAA, our finding that the transcriptional level of Dlk1 is markedly increased in MDS CD34+ cells is consistent with the results observed by other investigators (6-8). To further explore the functional role of this gene in dysplatic hematopoiesis, we intend to concentrate our study on Dlk1 after confirming its expression pattern in a larger patient cohort.

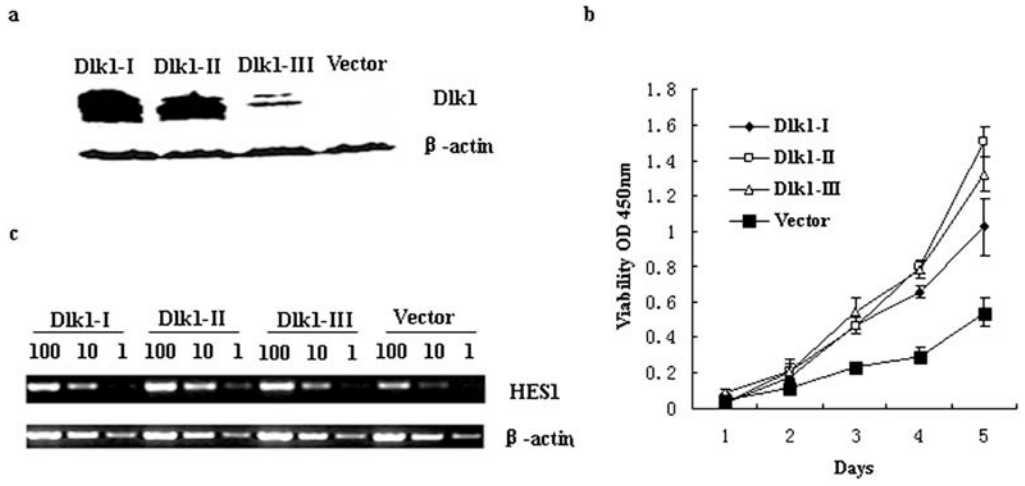


Figure 2. Dlk1 caused cellular proliferation of K562 cells. (a) Three stable clones expressing Dlk1 and control clones (transfer vector) of K562 cells were detected using Western blot analysis with anti-Dlk1 and anti-actin antibody. (b) Viability of three stable clones expressing Dlk1 and control clones (transfer vector) of K562 cells were detected by CCK-8. (c) HES1 and ß-actin were detected by RT-PCR in three stable clones expressing Dlk1 and control clones. 100, undiluted cDNA; 10, cDNA diluted 1:10; 1 1:100 dilution.

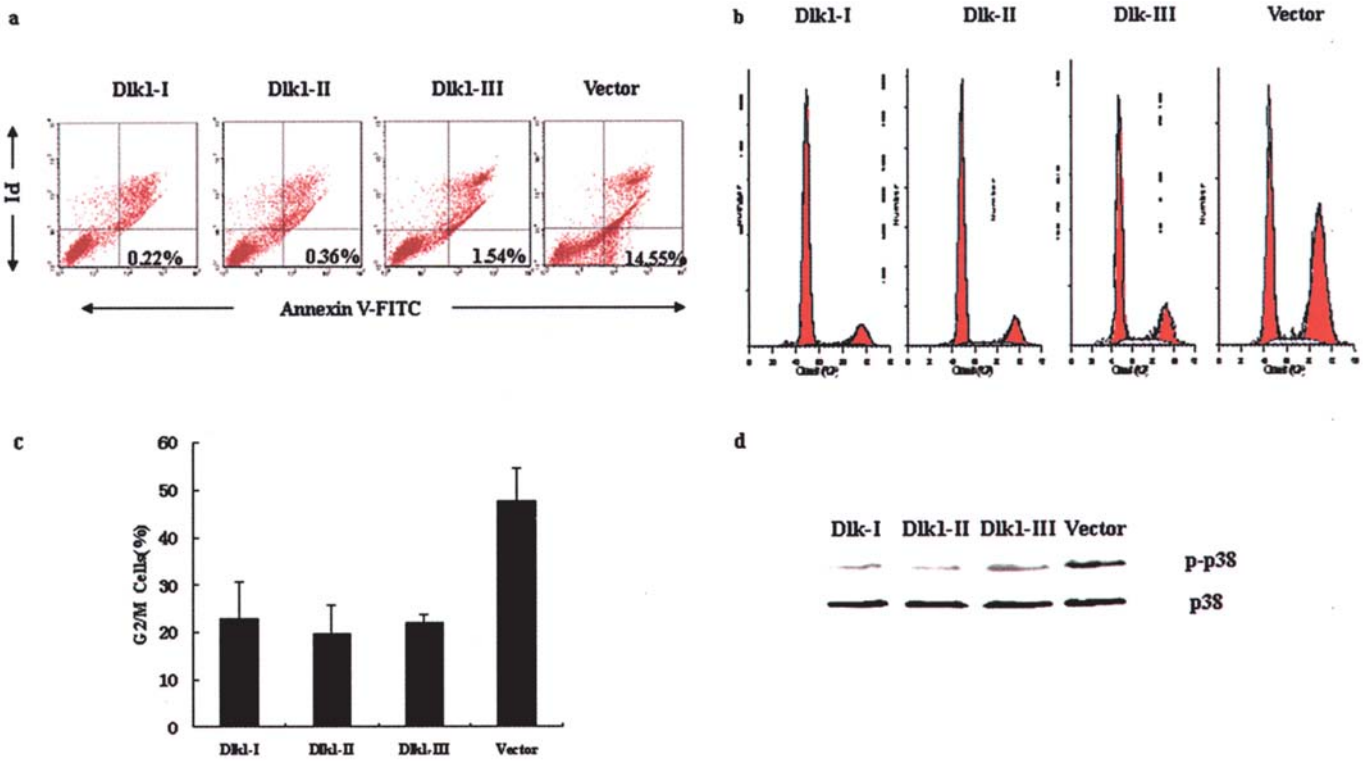


Figure 3. Dlk1 affects apoptosis induced by As_2O_3, and affects cell cycles induced by TPA. (a) Dlk1 affects apoptosis induced by As_2O_3. Cells were analyzed by flow cytometry. Annexin-V binds to cells expressing phosphotidylserine on the outer layer of the cell membrane, and PI stains the cellular DNA of cells with a compromised cell membrane. This allows for the discrimination of live cells (unstained with either fluorochrome) from apoptotic cells (stained only with annexin-V) and necrotic cells (stained with both annexin-V and PI). (b) Dlk1 affects cell cycles induced by TPA. Cells were analyzed by flow cytometry (FACScan). (c) Results of three experiments showing G2/M cells induced by TPA. (d) Western blot showing markedly reduced phosphorylated-p38 protein induced by TPA in three stable clones expressing Dlk1, compared to control clones (transfer vector) of K562 cells, with no difference in the p38 protein levels of these two groups.

Dlk1 (Pref-1, FA1 and pG2) known as an imprinted gene residing on chromosome 14, codes for a transmembrane and secreted protein (10). It contains a signal peptide belonging to the epidermal growth factor-like family, homologous to Notch/Delta/Serrate. Dlk1 protein has six epidermal growth factor-like repeats, a transmembrane domain, and a short intracellular tail (10), but lacks the DSL motif. In the mouse, this gene is transcribed only from the paternal allele. It contains a completely unmethylated CpG island promoter and an exonic differentially methylated region (DMR) which is hypomethylated on the maternal allele and partially methylated on the paternal allele.

The relevance of the Dlk1 gene to hematopoiesis was not studied until recently. In this study, we found that the expres-

lk1 was frequently up-regulated in MDS patients in on to nonleukemic control subjects. Although the mechanism underlying the overexpression of Dlk1 in MDS is not known, loss of imprinting led by aberrant epigenetic regulation of the gene may be a factor (15).

Although the intrinsic Dlk1 gene is transcribed at a relatively higher level in K562 human leukemia cells compared to other leukemia cell lines, its protein product can hardly be detected by Western blot analysis. The forced expression of Dlk1 in K562 cells transfected with exogenous Dlk1 enhanced their proliferation. This may be due to the increased level of HES1 which was up-regulated by the overexpressed Dlk1. Nevertheless, we and other investigators failed to detect any changes in $p21^{WAF1}$ by Western blot analysis. The expression of HES1 in Dlk1 transfected K562 cells was up-regulated whereas no change was found with regard to the Notch receptors. This may be a result of the lack of a DSL motif in Dlk1, which is considered to be crucial for interaction with Notch family molecules (12), suggesting that Dlk1 may exert its activity independent of the Notch receptors. The mechanism by which Dlk1 enhances cellular proliferation and protects cells from apoptosis induced by As_2O_3 remains to be further explored.

Li and colleagues had demonstrated that the overexpression of Dlk1 can inhibit the proliferation of HL-60 leukemic cells and impair the cell differentiation induced by ATRA (16), while Sakajiri *et al* showed the forced expression of Dlk1 enhanced the proliferation of K562 cells (17). Sakajiri's group also demonstrated that Dlk1 mRNA was highly expressed in other erythroleukemia and megakaryocytic leukemia cell lines resembling the M_6 and M_7 subtypes of AML (17). Our results were consistent with the latter study. Upon induction by TPA, more nontransfected control cells were arrested in G2/M phase, while three K562 stable transfectant clones overexpressing Dlk1 remained without changes in cell cycle and displayed less differentiation. These observations seem to indicate that Dlk1 is expressed in a hematopoietic lineage-specific manner and its expression may correlate with the determination of lineage differentiation.

Mammalian MAPKs are classified into three subfamilies: extracellular signal-regulated kinase, c-jun N-terminal kinase (JNK), or stress-activated protein kinase (SAPK), and p38MAPK. In development, complex cross-talk between Notch and the ras/MAPK pathway is well established in both invertebrates and vertebrates. Kim and colleagues showed that the active form of Notch1 functions as a negative regulator of JNK signaling in mouse cells (21) while Haruki *et al* reported that Notch3 induced MAPK phosphorylation and down-regulated MKP-1 in tumor cells (22). To further explore the interaction between Dlk1/Notch signaling pathway and the MAP kinase signaling pathway, we found that phosphorylated-p38 was markedly reduced in three stable Dlk1 transfectant K562 cell clones compared with control cells, after exposure to TPA. Nevertheless, no changes could be detected in the levels of phosphorylated-ERK and phosphorylated-JNK (data not shown), suggesting that the change in phosphorylated-p38 levels may partly mediate the action of Dlk1 on TPA induced cell differentiation.

In summary, we found an up-regulated expression of Dlk1 in MDS patients from the gene expression profiles obtained by microarray. In addition, numerous other genes were also found exhibiting differential expression patterns between MDS and CAA patients. The forced overexpression of the Dlk1 gene in transfected K562 cells can enhance cellular proliferation, inhibit cell apoptosis and affect cell cycle. We propose that the hematopoietic cell clones in MDS which overexpress Dlk1 might obtain a growth advantage through these effects and affect normal hematopoietic cells. Dlk1 may mimick Notch signaling while being somehow independent from this pathway. The effect of Dlk1 may be exerted through interaction with the MAP kinase pathway. In conclusion, the up-regulation of Dlk1 expression might be an important event in dysplastic hematopoiesis in human myelodysplastic syndrome.

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