REVIEW ARTICLE

EVI1 and its Role in Myelodysplastic Syndrome, Myeloid Leukemia and Other Malignant Diseases

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SUMMARY

Fuchs O.: EVI1 and its Role in Myelodysplastic Syndrome, Myeloid Leukemia and Other Malignant Diseases
The **e**cotropic **v**iral **i**ntegration site 1 (*EVI1*) gene was identified as a common locus of retroviral integration in myeloid tumors found in mice. The *EVI1* gene is highly conserved through evolution and human gene *EVI1* on chromosome 3q26 encodes zinc fingers-containing transcription factor. EVI1 is expressed in nonhematopoietic tissues but not in normal blood or bone marrow. EVI1 was detected in hematopoietic cells in retrovirus-induced myeloid leukemias in mice, and several reports documented EVI1 expression in human myelodysplastic syndromes and other hematologic malignancies without 3q26 translocations. EVI1 is abnormally expressed in human myeloid leukemias that are associated with the t(3;3)(q21;q26), t(3;21)(q26;q22), inv(3)(q21q26) and other chromosomal rearrangements. EVI1 is overexpressed in some ovarian cancers and human colon cancer cell lines and may play a role in the initiation and/or progression of solid tumors, as well as hematopoietic malignancies. EVI1 is a transcriptional repressor which inhibits transforming growth factor beta (TGFß) family signaling by binding signal transducers (Smad proteins) and recruiting transcriptional corepressors. TGFß is an important regulator of proliferation, differentiation, apoptosis and migration of cells. EVI1 inhibits TGFß-mediated apoptosis. Knockdown of EVI1 function by small interference RNA increases the sensitivity of malignant cells to TGFß-mediated or other inducer-mediated apoptosis. Overexpressed EVI-1 blocks granulocyte and erythroid differentiation and possesses the ability of growth promotion in some types of cells. EVI1 functions in some cases as a transcriptional activator which stimulates, for example, GATA2 and GATA3 promoters. The study of EVI1 target genes will help to clarify the mechanism by which EVI1 upregulates cell proliferation, impairs cell differentiation, and induces cell transformation.
Key words: EVI1, myeloid leukemia, chromosomal rearrangements, colon cancer, transforming growth factor beta.
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EVI1 (ecotropic viral integration site 1) transcription factor is encoded by a gene, highly conserved through evolution (the mouse and human nucleotide and amino acid sequences are over 90% identical). EVI1 gene was originally identified as a common retroviral DNA integration sequence in murine myeloid tumors (1). EVI1 gene expression was determined during embryogenic development in mice (2). High EVI1 expression was detected in the urinary system and in the Műllerian ducts of the urogenital ridge, in the respiratory epithelium, nasal cavities, the heart and in the developing extremities. However, EVII expression is very low in adults. Therefore EVI1 expression plays a role in organogenesis, cell proliferation and differentiation (2).

Targeted mutagenesis, resulting in a loss of functional EVI1 murine embryonic stem cells, was employed to assess the role of EVI1 in embryogenesis (3). Homozygous embryos missing EVI1 functionality presented with various defects and did not survive Day E 10.5. There is little information on the normal role of EVI-1. Most data were collected from studies on cell lines, where insertion of strong promoters enhance the *EVI1* gene expression (4, 5). EVI-1 facilitates expression of endogenous c-Jun and c-Fos, thus activating AP-1 transcription factor in NIH-3T3 and P19 cell lines. However, the effect of EVI-1 is indirect, because EVI-1 does not bind to the c-Jun and c-Fos gene promoters (4). Through its N-terminal zinc-finger site, the EVI1 binds directly to promoters of endogenous EVI1 target genes (6) or to the target genes sequence (6). So far, several such EVI1 target genes (*GATA2, GATA3, Gadd45g, SnoN* and other genes) have been described (6). The EVI1 target genes may play a role in EVI1-mediated cell transformation. EVI-1 protects cells from stress-induced apoptosis (5). The mechanism is based on the inhibition of c-Jun N-terminal kinase (JNK), a member of the mitogen-activated kinases family (MAPK). The above EVI-1 effects (including enhancement of the endogenous c-Jun and c-Fos expression and JNK inhibition) are based on EVI-1 molecule zinc-fingers loci, facilitating specific protein-protein interactions (4, 5). They include interaction between EVI-1 and several histone deacetylases, resulting in chromatin condensation and thus inhibition of EVI-1 target genes transcription (7). Interaction of EVI-1 and another transcription repressor – CtBP protein (**C**-**t**erminal **b**inding **p**rotein) enhances the EVI1 activity in EVI-1 target genes transcription inhibition (8). EVI1 also interacts with BRG1 protein (**br**ahma **r**elated **g**ene 1), a member of the SWI/SNF chromatin remodelling complex and a retinoblastoma protein regulator (pRB) – tumor suppressor, cell cycle (phase G1 to phase S cell transition) regulator. pRB – mediated inhibition of cell

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Table 1. Chromosomal rearrangements including 3q26.2 chromosome

Chromosomal translocations	disorder	EVI1 expression	fusion protein
t(2;3)(p13;q26)	CML-BK t-MDS	+	not detected
inv(3)(q21q26)	MDS, AML CML	+	riboforin-EVI1
t(3;3)(q21;q26)	MDS, AML CML	+	riboforin-EVI1
t(3;7)(q26;q21)	CML-BK AML	+	not detected
t(3;7)(q27;q22)	AML	+	not detected
t(3;12(p26;p13)	MDS, AML	+	TEL-MDS1/EVI1
t(3;13)(q26;q13-14)	AML	+	not detected
t(3;17)(q26;q22)	MDS	+	not detected
t(3;21)(q26;q22)	CML-BK AML, t-MDS	+	RUNX1-MDS1/EVI1

growth depends on regulation of the E2F transcription factors family. E2F activity is essential for expression of genes included in the phase G1 to phase S cell cycle transition and for DNA replication. EVI1 – protein BRG1 interaction activates E2F1 gene promoter, resulting in a faster cell cycle (9).

Chromosomes translocations including the *EVI1* gene (Table 1) and *EVI1* gene expression are involved in the myelodysplastic syndrome (MDS) and myeloid leukemia (10-14) pathogenesis. Increased EVI1 also plays a role in epithelial carcinomas, colorectal carcinomas and some ovarian carcinomas (15).

Partly, the oncogenic effect is based on EVI1 ability to block the cell growth inhibition effects of several cytokines, thus eliminating effects of interferon α (IFN-α) and TGF-ß through different mechanisms. Conversely, MDS1-EVI1 fusion protein enhances cells susceptibility to both cytokines, facilitating cell growth inhibition.

EVI1 also inhibits induction of the PML proapoptotic gene (encoding a promyelocytic leukemia protein).

EVI1 GENE AND PROTEIN STRUCTURE

EVI1 gene is located on chromosome 3 in the q26.2 region. It spans approximately 100 kb and it contains 12 exons (Fig. 1). Translation initiation codon is located in exon 3 (marked with an arrow on Figure 1). EVI1 is a nuclear protein consisting of 1051 amino acid residues and of molecular weight 145 kDa. The protein contains two zinc- finger domains (the one closer to the N-terminal contains 7 zinc-finger motives and the other, closer to the C-terminal, contains 3 zinc-finger, Cys_2His_2 type motives). EVI1 has an alternative, cut form of molecular weight 88 kDa (Fig. 2). This EVI1 form lacks zinc fingers 6 and 7 and its expression is regulated independently (16). EVI1 also contains a region rich in proline residues, located between the both zinc-finger regions and a region of amino acid residues at the C-terminal. The proline-rich region inhibits transcription of EVI1 target genes, whereas there is no evidence of the acidic region participating in transcriptional regulation.

MDS1- EVI1 GENE AND PROTEIN STRUCTURE

MDS1 (myelodysplasia syndrome 1) gene encodes a small protein (Fig. 1, 2), originally detected in the *AML1-MDS1-EVI1* fusion gene (17). The fusion gene resulted from t(3;21) chromosomal translocation. *MDS1* was mapped 150–300 kb proximal to the first *EVI1* exon on chromosome 3q26.2. Northern blot analysis (demonstration of a specific mRNA within a whole RNA, separated on an agarose gel under denaturing conditions and reblotted from the gel to nitrocellulose or other membrane, which was subsequently hybridized with a specific radioactively or fluorescently labeled specific cDNA probe) was performed with a specific MDS1 cDNA probe in various tissues. This specific probe detected transcripts of molecular weight 5.8 and 6.2 kb, corresponding to EVI1 transcripts detected with the cDNA probe (17). However, the MDS1-specific cDNA probe, compared to the EVI1 cDNA probe, detected further three transcripts spanning 1.0; 1.5 and 2.0 kb. Expression of the *MDS1-EVI1* fusion gene is independent on the *EVI1* gene transcription and is easily detectable in normal pancreatic and kidney tissues (17). MDS1-EVI1 fusion protein contains 188 amino acid residues, of which 125 are encoded by the first and second *MDS1* gene exon and the remaining 63 is encoded by the second exon and a nontranslated exon 3 sequence of the *EVI1* gene (17). The fusion protein region, resulting from transcription of the MDS exon 2 sequence and exon 2 and a nontranslated EVI1 exon 3 sequence, resembles so called PR domain in other proteins (PRDI-BF1 – **p**ositive **r**egulatory **d**omain **I**-**b**inding **f**actor **1**, RIZ1 – **r**etinoblastoma – **i**nteracting **z**inc finger protein **1**) (17). PRDI-BF1 and RIZ1 proteins, containing the PR domain, inhibit cell growth and induce cell differentiation. These proteins often mutate or exhibit low expression levels in tumor

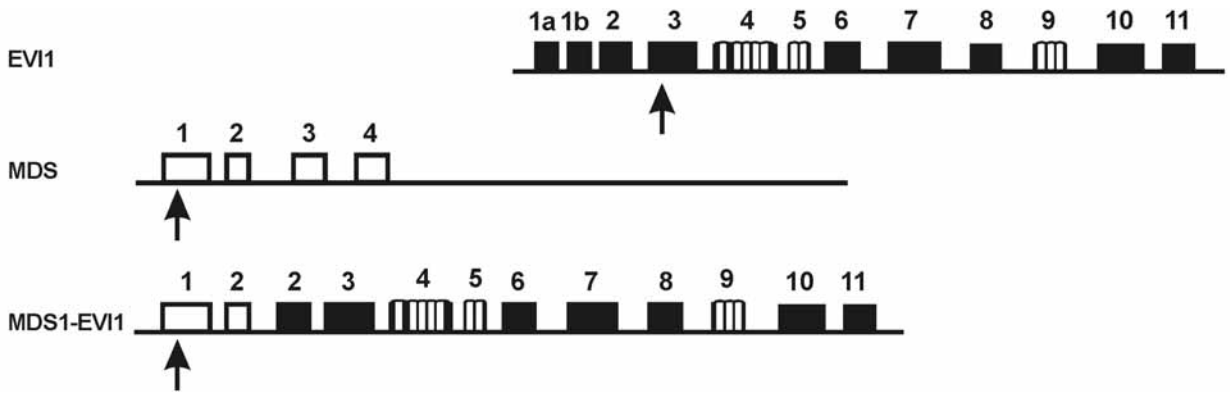

Figure 1. Structure of EVI1, MDS1 a MDS1-EVI1genes, which are included in the chromosome 3q26.2 rearrangements. A sequence corresponding to the translation initiation codon is indicated by an arrow. Individual exons are numbered.

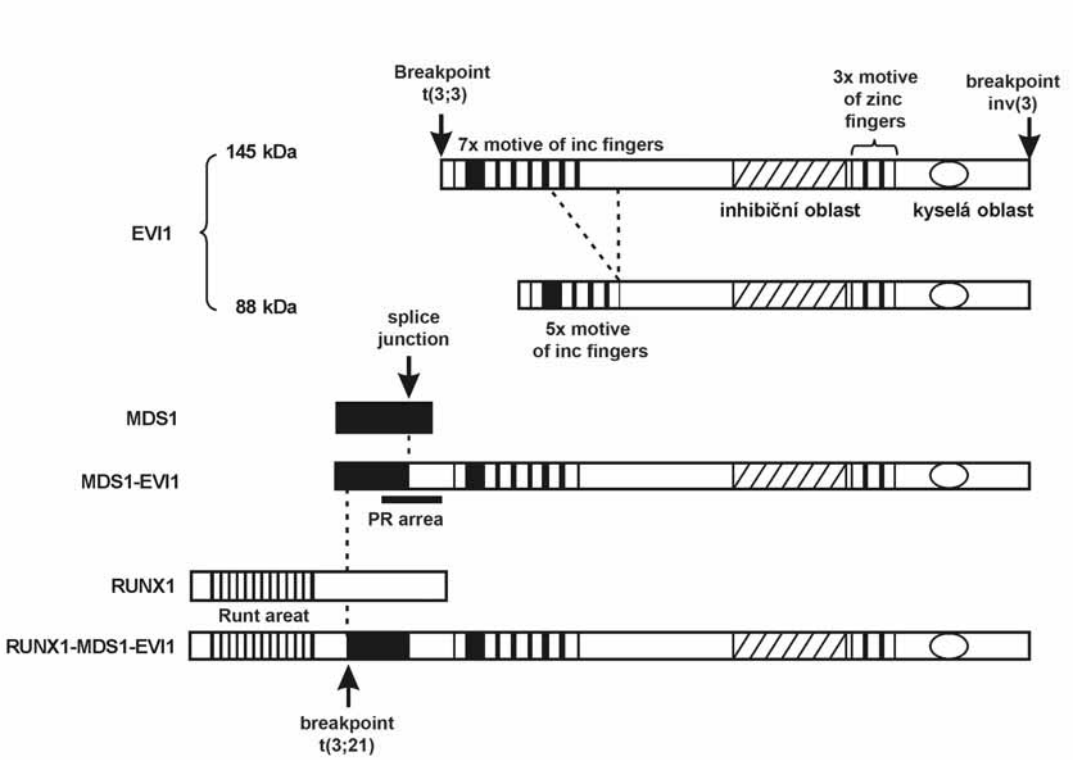

Figure 2. A schematic overview of EVI1, MDS1 and RUNX1 proteins structures and of MDS1-EVI1 and RUNX1-MDS1-EVI1 chimeric proteins Arrows point at places corresponding to breakpoints within individual chromosomal rearrangements. The dashed line indicates EVI1 splicing and the splice junction and chromosome rearrangements during RUNX1-MDS1-EVI1 fusion protein production. EVI1 exists in two forms, with different N-terminals. Individual significant protein regions are described in the text.

cells. On the contrary, EVI1 proteins and other proteins, expressed from the inner promoter and lacking the PR domain, exhibit enhanced expression levels in tumor cells and have oncogenic activity (18, 19).

EVI1 AND MDS1-EVI1 IN V HEMATOPOETIC CELL LINES AND IN BONE MARROW

EVI1 functions have been thoroughly studied in murine 32Dcl3 line hematopoetic cells, requiring interleukin 3 (IL-3) for their growth. Granulocyte colony stimulating growth factor (G-CSF) elicits differentiation of the cells into granulocytes. EVI1 – positive 32Dcl3 cells grow well in the IL3 presence, though their ability to respond to G-CSF deteriorates and they undergo apoptosis in the presence of G-CSF without IL3. EVI1 blocks expression of myeloperoxidase, granulocytic differentiation and apoptosis (20). The MDS1-EVI1 fusion protein has no effect on granulocytic differentiation of 32Dcl3 cells, though it has a strong inhibitory effect on their IL3-stimulated growth (20). Furthermore, the MDS1-EVI1 fusion protein inhibits growth of transgenic embryonic murine stem cells in *in vitro* differentiation and strongly reduces a number of differentiated hematopoetic colonies in semi-solid medium containing 1 % of methylcelulose (21). On the contrary, EVI1 is a potent growth stimulant of transgenic embryonic murine stem cells, increasing number of the colonies. EVI1 mostly stimulates differentiation into the megakaryocytic line, i.e. acetylcholine-positive colonies (21). The above finding, i.e. connection between enhanced EVI1 expression and abnormal megakaryopoesis, correlates with findings in hematologic disorders linked to chromosome 3q26 aberations.

Furthermore, EVI1 blocks erythroid differentiation, although not affecting expression of two main erythroid differentiation genes, i.e. erythropoietin and GATA1 transcription factor (22, 23). However, EVI1 blocks activation of the GATA1 transcription factor, thus affecting transcription of its target genes.

A ROLE OF EVI1 IN THE MYELODYSPLASTIC SYNDROME AND MYELOID LEUKEMIAS

EVI1 has not been detected in normal hematopoetic cells (14). *EVI1* gene expresssion in hematopoetic cells is associated with aggressive forms of MDS or AML, when the *EVI1* gene is activated following chromosome 3q26.2 rearrangement (19). *EVI1* gene expression may also be associated with deregulation of the *EVI1* gene promoter without chromosome 3q26.2 rearrangement (24), for instance in a fairly rare myeloproliferative disorder in childhood (JMML – **j**uvenile **m**yelo**m**onocytic **l**eukemia) (25). The commonest chromosome 3q26.2 rearrangements, associated with expression of *EVI1* or of a fusion gene, containing gene *EVI*, are summarized in Table 1. The inv(3)(q21q26) and t(3;3)(q21;26) rearrangements can be detected in 7–10 % of MDS/AML cases (26). They are associated with formation of *riboforin I-EVI1* fusion gene, which is controled by the strong promoter of riboforin I gene (27). The t(3;21)(q26;q22) chromosome rearrangements are associated with new as well as with therapy-derived MDS/AML cases and with some blast crisis chronic myeloid leukemia (CML) cases (28–31). The N-terminal region of RUNX1 containing the conserved Runt domain, a DNA binding site, makes a hybrid protein with MDS1/EVI1. The *RUNX1* gene encodes the α subunit of the heterodimeric transcription factor, known as CBF (**c**ore **b**inding **f**actor) or PEBP2 (**p**olyomavirus **e**nhancer-**b**inding **p**rotein

2). The *RUNX1* gene was originally marked as *AML1* (acute myeloid leukemia 1). The RUNX1 transcription factor binds to acetyltransferases (p300/CPB – **c**AMP responsive element **b**inding **p**rotein) and activates transcription of the granulocyte and macrophage colony stimulating factor (GM-CSF) and critical transcription factors in myeloid differentiation, e.g. PU.1, etc. The RUNX1-MDS1-EVI1 and RUNX1-EVI1 fusion proteins bind to class I (HDAC 1–3) histone deacetylases (HDAC) via transcription corepessor (**C**-terminal **b**inding **p**rotein) and inhibit transcription of the above RUNX1 transcription factor target genes, thus inhibiting differentiation of the myeloid progenitor cells (Fig. 3) (32).

C/EBPα transcription factor (**C**CAAT/**e**nhancer **b**inding protein α) is required for differentiation of granulocyte progenitors (33). RUNX1-MDS1-EVI1 fusion protein inhibits mRNA translation for the C/EBPα transcription factor, therefore, acting at the posttranscriptional level (33). Calreticulin, a potent inhibitor of the C/EBPα mRNA translation, is activated via the RUNX1-MDS1-EVI1 fusion protein (33).

Another mechanism of eliciting malignant transformation of hematopoetic stem cells, is inhibition of the transformation growth factor ß (TGF-ß) signaling pathway by the RUNX1-MDS1-EVI1 and RUNX1-EVI1 fusion proteins. EVI1 part of the RUNX1-MDS1-EVI1 and RUNX1-EVI1 chimer proteins binds the TGF-ß signal transmitter, the Smad3 protein, blocking its transcriptional activity for the TGF-ß target genes expression. (34–36). However, the MDS1-EVI1 hybrid protein stimulates the TGF-ß signaling path (35).

The fourth mechanism, participating in malignant transformation of hematopoetic stem cells, is inhibition of c-Jun N-terminal kinase activity (JNK). JNK inhibition blocks stress-induced cell apoptosis.

The fifth mechanism is boosting the AP-1 transcription factor activity by the RUNX1-EVI1 fusion protein-mediated activation of the c-Fos gene promoter. All the above five mechanisms participate in malignant transformation of hematopoetic stem cells (29, 33).

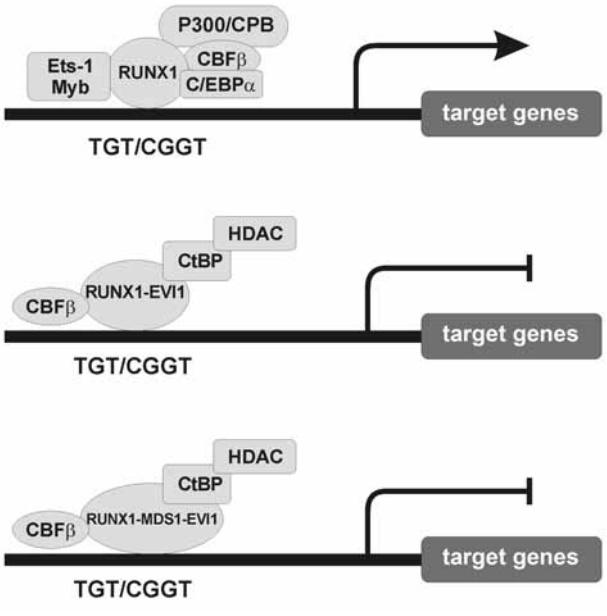

Figure 3. RUNX1 - CBFß complex acts as RUNX1 target genes transcription activator, following interactions with other specific transcription factors, e.g. with Ets-1/Myb, C/EBPα and with acetyltransferase P300/CBP.
Contrary to that, RUNX1-EVI1 a RUNX1-MDS1-EVI1 fusion proteins act as RUNX1 target genes transcription supressors, as they bind to the CtBP transcription cosupressor and histone deacetylase. A detailed description is provided in the text.

The *RUNX1-MDS1-EVI1* fusion gene role has also been studied *in vivo.* Retroviral transduction was used to induce expression of the *RUNX1-MDS1-EVI1* fusion gene in murine bone marrow cells. Transplantation of the cells with the *RUNX1-MDS1-EVI1* fusion gene expression to mice resulted in AML 5–13 months following the procedure (37). RUNX1-MDS1-EVI1 co-acts e.g. with the bcr-abl fusion protein, originating from t(9;22)(q34;q11) chromosome rearrangement, to induce AML. Neither EVI1, nor the MDS1-EVI1 fusion protein, on their own, even in the bcr-abl presence, are able to induce AML in mice.

Reduced expression of *SCL* (encoding helix-loop-helix transcription factor mRNA) and *LMO2* (encoding nuclear protein mRNA with LIM region containing a double zinc-fingers motif) genes is thought to play a role in malignant transformation of hematopoetic cells, as well. Expression of both the above mentioned genes is required for embryonic erythropoiesis. Reduced expression of the genes has been observed in murine embryos with the *RUNX1-EVI1* chimer gene (38).

A ROLE OF EVI1 IN NONHEMATOLOGICAL MALIGNANCIES

Enhanced expression of EVI1 plays a role in other malignancies, e.g. in some ovarian carcinomas and colorectal carcinoma (15). EVI1 induces cell resistance against apoptosis (15). The TGF β – mediated antiapoptotic resistance mechanism is based on activation of phosphatidylinositid-3-kinase (PI3K) signaling pathway and on phosphorylation of serin-threonin protein kinase Akt, also called protein kinase B (PKB) (15). Activated protein kinase Akt/PKB affects proteins included in apoptosis, such as bcl-2 family member, Bad, caspase 9, transcription factor "forkhead" and mdm2 (according to its position on the **m**urine **d**ouble **m**inute chromosome **2**). Activated protein kinase Akt also phosphorylates the IKKα (IκB kinase α) protein, which further phosphorylates IκB (NF-κB transcription factor inhibitor), thus activating the NF-κB transcription factor. Activated NF-κB then may transfer into the cell nucleus, where it controls target genes transcription of a number of antiapoptotic proteins (e.g. bcl 2, bcl-XL, cell inhibitors of apoptosis c-IAP1, c-IAP2 and others).

CONCLUSION

EVI1 expression was detected in blast crisis MDS, AML and CML in blast crisis and results from chromosome rearrangements, as well as from abnormal activation of a respective gene. Enhanced expression of EVI1 also plays a role in other malignancies – e.g. in ovarian carcinomas and colorectal carcinoma (15). Nowadays, large study groups of bone marrow specimens from AML patients are analysed for expression of a great number of genes, including the *EVI1* gene, using microchip techniques (e.g. Affymetrix GeneChip arrays), and the results show that findings of high EVI1 mRNA levels are prognostically unfavourable in patients with MDS and AML (19), which corresponds with the results from former studies, using the RT-PCR or Northern blot methods (19). The MDS patients suffer from increased thrombocyte counts, significant megakaryocyte hyperplasia and dysplasia, and anemia. The patients would be elderly and, frequently, bone marrow transplantation cannot be considered, therefore, significant efforts are devoted to the prospective use of efficient farmacotherapy. Clinical studies have shown that administration of arsenic trioxide (As_2O_3) in combination with thalidomide is very efficient (39, 40). Arsenic trioxide (Trisenox) has been successfully used in acute promyelocytic leukemia (APL).

Abbreviations

Akt – proteinkinase phosphorylating serine and threonine residues
AML – acute myeloid leukemia
AP1 – transcription factor, heterodimer consisting of c-fos and c-jun protooncogenes
Bad – proapoptotic protein containing BH3 region and included in the bcl-2 protein family
bcl-2 – antiapoptotic protein (B cell CLL/lymphoma 2)
bcl-XL – antiapoptotic protein included in the bcl-2 protein family
BRG1 – brahma related gene 1
CBF – core binding factor
CBP – cAMP responsive element binding protein
C/EBPα – CCAAT/enhancer binding protein α
c-IAP – cellular inhibitor of apoptosis
CML – chronic myeloid leukemia
CtBP – C–terminal binding protein
E2F – transcription factor required for expression of genes included in G1/S cell cycle transition and for DNA replication
EVI1 – ecotropic viral integration site 1
GADD45g – growth arrest and DNA-damage-inducible, gama
GATA – family of six transcription factors (1–6), the first three of them are located in hematopoietic cells and play a role in cell cycle differentiation and regulation
G-CSF – granulocyte -colony stimulating growth factor
HDAC – histone deacetylase
IFNα – interferon α
IL3 – interleukin 3
JMML – juvenile myelomonocytic leukemia
JNK – c-Jun N-terminal kinase
MAPK – mitogen-activated protein kinases
mdm2 – murine double minute 2, ubiquitin ligase, regulating p53 by binding to its transactivation region, inactivating it. The process of ubiquitinylation flags it for destruction.
MDS – myelodysplastic syndrome
NF-κB – nuclear transcription factor
PEBP2 – polyomavirus enhancer binding protein 2
pRB – retinoblastoma protein
PRDI-BF1 – positive regulatory domain I-binding protein 2
RIZ1 – retinoblastoma-interacting zinc finger protein 1
Smad – transcription factors family included in TGF–ß cytokines signal transduction
SnoN – Ski related novel gene, transcription corepressor
SWI/SNF – chromatin- remodelling complex
TGF-ß – transformation growth factor beta
t-MDS – therapy-derived MDS cases

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