

Molecular Pathogenesis of MDS

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Abstract

Myelodysplastic syndromes (MDS) are considered to be a family of clonal disorders of hematopoietic stem cells that are characterized by ineffective hematopoiesis and susceptibility to acute myelogenous leukemias, and are shown to be strikingly refractory to current therapeutic modalities. A substantial proportion of these complex diseases arise in the setting of exposures to environmental or occupational toxins, including cytotoxic therapy for a prior malignancy or other disorder. The conversion of a normal stem cell into a preleukemic and ultimately leukemic state is a multistep process requiring the accumulation of a number of genetic lesions. On the genomic level, MDS is typified by losses and translocations involving certain key gene segments, with disruption of the normal structure and function of genes that control the balance of proliferation and differentiation of hematopoietic precursors. More than a half of the chromosomal abnormalities in MDS comprise deletions of chromosomes 5, 7, 11, 12, 13 and 20. This evidence suggests that as yet unidentified tumor suppressor genes should have important roles in the molecular mechanisms of MDS. Further molecular approaches to such genetic lesions will identify the relevant tumor suppressor genes. Over the past years, major signal transduction molecules were identified and their genetic alterations were extensively analyzed in MDS as well as leukemias. These include receptors for growth factors, RAS signaling molecules, cell cycle regulators, and transcription factors. Among them, notable is transcription factors that regulate both proliferation and differentiation of hematopoietic stem cells. The disruption of the normal flow of the signal transduction pathways involving these molecules translates into ineffective multilineage hematopoiesis and bone marrow failure. Therefore, MDS provides a fertile testing ground on which we could study the molecular dissection implicated in the multistep leukemogenesis.

Key Words: Myelodysplastic syndrome; Genetic lesion; Chromosomal abnormality

1. Biology

Myelodysplastic syndrome (MDS) is a family of clonal disorders characterized by dysmyelopoiesis and susceptibility to acute myelogenous leukemia (AML). The natural history of these diseases ranges from a chronic course that may span years to a rapid course toward leukemic progression. Clonal proliferation is the consequence of acquired somatic mutation that confers a proliferative advantage to cells. Identification of clonal cells provides valuable information on the molecular pathogenesis of the disease, the processes that govern the transition to AML, and diagnostic and prognostic information that is useful in the clinical approach to MDS. There are at least several methods for determining clonality of cells; cytogenetic analysis and fluorescence *in-situ* hybridization (FISH) analysis based on

chromosomal abnormalities, Southern blot analysis and single strand conformation polymorphism (SSCP) analysis based on genetic alterations, and X-inactivation-based clonality analysis.

Clinically, patients with MDS present with variable cytopenias owing to an ineffective hematopoiesis of unknown etiology. An important recent observation in this regard is the excessive intramedullary apoptotic death seen in the bone marrow biopsies of these patients, possibly accounting for the invariable cytopenias. One mechanism invoked to explain the apparent discrepancy between cellular marrow and peripheral blood cytopenias in patients with MDS is programmed cell death (apoptosis), which occurs with increased frequency in MDS marrow [1,2]. Several cytokines or ligands known to have proapoptotic properties, such as interleukin-1 β (IL-1 β), tumor necrosis factor α (TNF α), and Fas-ligand

are up-regulated in many patients with MDS [1,3]. It is shown that blockade of TNF α or Fas-ligand enhances hematopoietic colony transformation from MDS marrow in vitro and improves blood cell counts in vivo [1]. However, it is clear from those studies that the regulation of hematopoiesis in MDS is complex and multiple factors are involved.

Hematopoietic stem cells of MDS are genetically unstable and thus susceptible to genetic lesions. Once a stem cell in MDS gains a dominant mutation over the normal cell growth, such a cell shows the clonal evolution to become much more susceptible to further multiple genetic mutations, and finally develops to leukemia. The initial genetic event of the presumed multistep pathogenesis of MDS is an unknown stem cell defect, which is followed by non-clonal karyotype instability. In approximately half of the patients, clonal karyotypic abnormalities, which are detectable at the stem cell level, occur [4]. By unravelling loss of heterozygosity (LOH) and microsatellite instability (MSI), microsatellite polymorphic markers are useful to test the status of DNA mismatch repair (MMR) and cell cycle-conducting tumor-suppressor genes, both of which, if deficient, promote genetic instability [5].

LOH describes the homozygous state of a distinct chromosomal region and points to the presence of a closely located inactivated tumor-suppressor gene that might be involved in malignant transformation. MSI is defined by the occurrence of novel microsatellite alleles in neoplastic DNA when compared with DNA from non-malignant tissue of the same individual. It is a hallmark of patients with hereditary non-polyposis colorectal cancer (HNPCC) and related malignancies as the consequence of a defective DNA MMR machinery [6]. MSI has also been observed in sporadic tumors and distinct hematological disorders, suggesting that DNA proofreading failure may be a common pathogenetic mechanism in neoplastic transformation. A disease-inherent genetic instability is thus supposed to be the basis for an accumulation of somatic mutations in MDS.

2. Cytogenetics and Genetic Alterations

Cytogenetic analysis is a cornerstone for characterization of MDS, and provides valuable clues to the molecular pathogenesis of MDS. Since more than 70% of patients with MDS have clonal cytogenetic abnormalities, cytogenetic studies play a pivotal role in defining the concept of primary MDS and therapy-related MDS, establishing the diagnosis, evaluating prognosis for survival and transformation to AML, and approaching to molecular basis of the diseases. Some patients with MDS exhibit aneuploidy, in which a whole chromosome is either lost or gained, or structural abnormalities such as deletions, translocations, isochromosomes or marker chromosomes. The complexity of abnormal karyotypes is more common in therapy-related MDS than in primary MDS [7,8].

2.1. Chromosomal Deletions

One of the most prominent characteristics in chromosomal abnormalities observed in MDS is a predominance of chromosomal deletions, whereas de novo leukemias are typified by balanced reciprocal translocations. Frequent loss of genetic loci leads to the hypothesis that MDS may be caused by inactivation of tumor suppressor genes. Based on this hypothetical model, one copy of a gene is deleted by chromosomal deletion, and the other copy is inactivated by point mutation, minute deletion, or loss of expression due to regional methylation. So far, however, this paradigm for MDS has not been proven, probably because of the difficulty in cloning tumor suppressor genes within the large range of chromosomal deletions.

2.1.1. 5q-

The 5q- chromosomal abnormality is the most frequent in MDS, and present in more than 20% of MDS patients. The 5q- syndrome is characterized by distinct clinical and morphological features including refractory macrocytic anemia with dyserythropoiesis, high prevalence in female, normal or high platelet count, small megakaryocytes, and relative good risk [9]. The breakpoints within a large region of 5q are highly variable among patients, but the most critical region of deletion is supposed to lie between 5q31 and 5q33 [10]. Several genes encoding hemopoietic growth factors and receptors, comprising IL-3, IL-4, IL-5, M-CSF (CSF-1), GM-CSF, and the receptor for M-CSF (CSF-1R), are localized to the long arm of chromosome 5, and there has been much speculation that deletion of one or more of these genes may be critical to the pathogenesis of the associated MDS [11]. The homozygous loss of some of these genes has still been considered as a possible mechanism in the pathogenesis of myeloid disorders with 5q deletion. IRF-1, a gene whose product manifests anti-oncogenic activity, is mapped to 5q31.1. IRF-1 lies between IL-5 and CDC25C and is centromeric to IL-3 and GM-CSF. Among these genes, IRF-1 is reported to be deleted at one or both alleles by an accelerated exon skipping mechanism in some cases of MDS with aberrations of 5q31 [12]. Pur α is a highly conserved, eukaryotic sequence-specific DNA- and RNA-binding protein involved in diverse cellular and viral functions including transcription, replication, and cell growth. PURA gene, encoding Pur α , is localized to chromosome bands 5q31.1 and is shown to be hemizygotously deleted in MDS and AML. Frequent deletion of PURA indicates that PURA is one of the most commonly deleted genes in myeloid disorders characterized by del(5)(q31) [13].

Another approach to identify a target gene, which is inactivated by 5q deletion, is to search a gene that might be also target for chromosomal translocations involving a region between 5q31 and 5q33. There have been several such genes so far cloned. The NPM-MLF1 chimeric protein is produced by the t(3;5)(q25.1;q34)

chromosomal translocation, which is associated with MDS prior to progression into AML [14]. Leukemia cells ectopically expressing NPM-MLF1, but not those with wild-type NPM or MLF1 are shown to undergo apoptosis. GRAF gene (for GTPase regulator associated with the focal adhesion kinase pp125FAK), which encodes a member of the Rho family of the GTPase-activating protein, is revealed to fuse with MLL in a unique t(5;11)(q31;q23) that occurred in an infant with juvenile myelomonocytic leukemia [15]. The particular position of the human GRAF gene at 5q31 and the proposed tumor suppressor properties of its avian homologue suggest that it also might be pathogenetically relevant for hematologic malignancies with deletions of 5q. Moreover, some mutations within the GAP domain of the second GRAF allele resulting in inactivation of both alleles in at least some cases suggests that deletions and mutations of the GRAF gene may be instrumental in the development and progression of hematopoietic disorders with a del(5q). Other than these genes, there also exist more genes including acyl CoA synthetase 2 gene, ACS2, fused to TEL gene in t(5;12)(q31;p13) translocation in patients with MDS [16], and nuclear receptor-binding SET-domain-containing protein, NSD1, fused to NUP98 in t(5;11)(q35;p15.5) translocation associated with 5q deletion in childhood AML [17].

2.1.2. Monosomy 7

Monosomy 7 and 7q- are among the most frequent chromosomal abnormalities in MDS, and are associated with poor prognosis in terms of either short survival or leukemic evolution. Although genes on chromosome 7 that are responsible for the disease phenotype are not identified, a critical region at 7q22.1 has been clarified [18]. A potential myeloid tumor suppressor gene, PIK3CG, has recently been identified in this locus, but is unlikely to act as a recessive tumor suppressor gene in MDS with monosomy 7 [19]. Monosomy 7 is also found in children with juvenile myelomonocytic leukemia (JMML), which has been referred to as monosomy 7 syndrome. JMML is a pediatric myelodysplastic syndrome that is associated with neurofibromatosis type 1 (NF1). The NF1 tumor suppressor gene encodes neurofibromin, a GTPase-activating protein (GAP) for p21RAS. Children with NF1 are predisposed to JMML, and both alleles of the NF1 gene are inactivated in leukemic cells in some patients with NF1 [20]. NF1 gene mutations have been detected in approximately 30% of JMML cases. RAS gene mutations or inactivation of NF1 gene are thought to be critical events in the progression of MDS with Monosomy 7 [21,22]. In conjunction with monosomy 7, it is under investigation whether unbalanced translocation, [-7, +der(1;7)(q10;p10)], which is frequently detected in MDS, is biologically the same as monosomy 7.

2.1.3. 20q-

A chromosomal 20q deletion is associated with ap-

proximately 5% of primary MDS and confers a relatively favorable prognosis. Erythrocytic and megakaryocytic lineages appear to be mainly involved in dysplastic changes. In some MDS cases with 20q-, clonal mature granulocytes from peripheral blood lack the anomaly. An increased bone marrow apoptosis of granulocytic precursors bearing 20q- may support these findings [23]. Deletions are always interstitial and the crucial region deleted in MDS has been mapped between D20S174 and D20S17. Although the deletion is still quite large by molecular standards, several candidate tumor suppressor genes are mapped to this locus [24].

2.1.4. Less Frequent Deletions

In addition, a number of less frequent but characteristic loss of a part or whole of a chromosome have been identified in MDS, including del(13q), del(11q), del(12p). Deletion of 13q consistently involve bands q14 and q21, and FISH analysis delineates a commonly deleted region flanked by YAC 833A2 and YAC 854D4 [25]. Much interest has been focused on RB gene, which is located at 13q14 and deleted in some MDS cases with del(13q). Some of MDS cases with a deletion of 11q are characterized by ringed sideroblasts, and are diagnosed as acquired sideroblastic anemia according to the FAB classification [26]. Genomic studies mapped the putative tumor suppressor gene to q22.2-q23.3 of chromosome 11. MDS cases with a deletion of 12p are heterogenous. Deletions are usually interstitial with loss of a region between bands p11 and p13, where KIP1 (CDKN1B) and TEL (ETV6) are located [27].

2.2. Chromosomal Translocation

2.2.1. TEL(ETV6) Fusion

A t(5;12)(q33;p13) translocation is a recurrent chromosomal abnormality in a subgroup of myeloid malignancies with features of both myeloproliferative disorder and MDS (Table 1) [28]. The molecular consequence of t(5;12) is a fusion between the platelet-derived growth factor receptor- β (PDGFR- β) gene on chromosome 5 and an ETS-like gene, TEL (ETV6), on chromosome 12. Eosinophilia and/or monocytosis in bone marrow are predominant morphological features in MDS with t(5;12) [29]. Oligomerization of TEL/PDGFR- β through the TEL HLH domain leads to constitutive activation of the PDGFR- β tyrosine kinase domain and thereby cellular transformation [30]. TEL gene is also fused to ARNT, MN1, EVI-1 and ACS2 in MDS cases carrying t(1;12)(q21;p13), t(12;22)(p13;q11), t(3;12)(q26;p13) and t(5;12)(q31;p13), respectively [31-34].

2.2.2. MLL Fusion

The human homolog of Drosophila trithorax gene, MLL, is a common target of chromosomal translocations involving 11q23, which are associated with acute

Table 1.

Chromosomal Translocation in MDS or MDS/AML.

TEL (ETV6) fusion	
t(1;12)(q21;p13)	TEL/ARNT
t(12;22)(p13;q11)	MN1/TEL
t(5;12)(q31;p13)	ACS2/ TEL
t(3;12)(q26;p13)	TEL/EVI-1
t(5;12)(q33;p13)	TEL/PDGFR-β
t(9;12)(q22;p12)	TEL/SYK
MLL fusion	
t(11;19)(q23;p13.1)	MLL/MEN(ELL)
t(5;11)(q31;q23)	MLL/GRAF
t(11;16)(q23;p13)	MLL/CBP
Nucleoporin abnormality	
t(7;11)(p15;p15)	NUP98/HOXA9
inv(11)(p15q22)	NUP98/DDX10
t(2;11)(q31;p15)	NUP98/HOXD13
t(11;17)(p15;q21)	NUP98/HOXB
t(11;12)(p15;q13)	NUP98/HOXC
t(11;20)(p15;q11)	NUP98/TOPI
t(6;9)(p23;q34)	DEK/CAN
EVI-1 family expression	
t(3;3)(q21;q26)	EVI-1 expression
inv(3)(q21q26)	EVI-1 expression
t(1;3)(p36;q21)	MEL1 expression
t(3;21)(q26;q22)	AML1/EVI-1
t(3;12)(q26;p13)	TEL/EVI-1
Others	
t(3;5)(q25.1;q34)	NPM/MLF1

leukemias showing a biphenotypic or a monocytic phenotype, infant leukemias, or topoisomerase inhibitor-induced secondary leukaemias [35]. Although the most typical 11q23 translocations such as t(4;11)(q21;q23), t(9;11)(q21;q23) and t(11;19)(q23;p13.3) are not found in primary MDS, some 11q23 translocations including t(11;19)(q23;p13.1) and t(11;16)(q23;p13), which generate MLL/MEN (ELL) and MLL/CBP chimeric genes, respectively, are detected in primary or therapy-related MDS [36,37]. Tandem duplication of the MLL gene is identified in some MDS with either normal karyotype or trisomy 11 [38].

2.2.3. Nucleoporin Abnormality

NUP98 is a nucleoporin, and is involved in nuclear import and export of proteins and RNAs [39]. To date, a number of chimeric NUP98 genes have been identified in patients with therapy-related AML or MDS with chromosomal translocations involving 11p15.5. The resultant chimeric transcripts encode fusion proteins that juxtapose the N-terminal GLFG repeats of NUP98 to the C-terminus of the partner gene. The chromosomal

aberrations, t(7;11)(p15;p15), t(2;11)(q31;p15), t(11;17)(p15;q21), t(11;12)(p15;q13), t(11;20)(p15;q11), and inv(11)(p15q22) result in generation of NUP98/HOXA9, NUP98/HOXD13, NUP98/HOXB, NUP98/HOXC, NUP98/TOPI, and NUP98/DDX10 fusion genes, respectively [40-44]. These results indicate that NUP98 is a recurrent target in therapy-related leukemias and MDS.

Translocation (6;9)(p23;q34) is a cytogenetic aberration that can be found in specific subtypes of both AML and MDS [45]. This translocation is associated with an unfavorable prognosis. CAN, also called NUP214, is a nucleoporin that contains multiple FG-peptide sequence motifs. It interacts at the nuclear pore complex with at least two other proteins, the nucleoporin NUP88 and hCRM1 (exportin 1), which was recently shown to function as a nuclear export receptor [46].

2.2.4. EVI-1 Family

Structural alterations involving 3q21 and 3q26 bands occur in approximately 2% of patients with AML or MDS. The major alterations are inv(3)(q21q26) and t(3;3)(q21;q26) and are classified as the 3q21q26 syndrome with features of abnormalities of megakaryocytopoiesis, an elevated platelet count, and poor prognosis [47]. Aberrant expression of the ecotropic virus integration-1 (EVI-1) gene, located at 3q26, is mediated by enhancer elements of the Ribophorin I gene, located at 3q21 [48]. The translocation t(1;3)(p36;q21) observed in a subset of MDS or AML, which is characterized by trilineage dysplasia, dysmegakaryocytopoiesis, and poor prognosis. In this abnormality, MEL1 gene, which is located at 1p36 and highly homologous to the EVI-1 gene, is transcriptionally activated by the translocation of the 3q21 region with the Ribophorin I gene [49]. The t(3;21)(q26;q22) translocation associated with therapy-related AML and MDS, and blastic crisis of CML generates the AML1/EVI-1 chimeric gene, resulting in aberrant expression of an almost whole coding region of EVI-1 protein [50]. As a mechanism of leukemogenesis induced by ectopic expression of EVI-1, EVI-1 is shown to repress Smad-induced transcription through recruiting a corepressor, C-terminal binding protein (CtBP), which binds to histone deacetylase (HDAC) [51,52].

2.3. Genetic Mutations

2.3.1. RAS Gene

RAS is an important signaling component for cell proliferation, and is activated by receptor tyrosine kinases (RTKs) stimulated with extracellular ligands [53]. RAS functions as a relay switch that is positioned downstream of cell surface receptor tyrosine kinases and upstream of a cytoplasmic cascade of kinases including the mitogen-activated protein kinases (MAPKs). Activated MAPKs in turn regulate the activities of nuclear transcription factors. RAS has the GTP-binding activity and the GTP-hydrolyzing property. RAS/GDP is an inactive form and is converted to an active form of

Table 2.

Genetic or Epigenetic Alterations in MDS.

RAS signaling molecules	
N-RAS (15-20%)	: point mutation
FLT3 (5%)	: tandem duplication
NF1 (rare)	: mutation
FMS (rare)	: mutation
KIT (rare)	: mutation
Cell cycle regulators	
p15 (30-50%)	: promoter methylation
p53 (5-10%)	: mutation, deletion
p16 (rare)	: mutation, deletion
RB (rare)	: mutation, deletion, promoter methylation
Transcription factors	
EVI-1 (30-40%)	: ectopic expression
IRF-1 (20-30%)	: exon skipping
AML1 (5%)	: mutation
C/EBP α (rare)	: mutation
WT1 (rare)	: mutation

RAS/GTP by guanine nucleotide-exchange protein (GEP) upon activation of RTKs with extracellular ligand stimuli. RAS/GTP interacts with target proteins such as RAF, thereby activates the downstream signaling molecules, MAPKs, and is converted to an inactive form RAS/GDP by hydrolysis of bound GTP in the presence of GTPase-activating protein (GAP). Mutated RAS proteins do not show GTPase activity, thus accumulate RAS/GTP, and thereby constitutively activate the downstream signaling.

RAS genes are known to be activated by point mutations at codon 12, 13 or 61 [54]. Among RAS genes, mutations of the N-ras gene are most frequent and detected in 20-30% of human leukemias and 10-15% of MDS cases (Table 2) [55]. An N-ras mutation in MDS is associated with short survival period and increased probability of developing AML [56]. These observations suggest that activation of N-ras oncogene should be related to leukemic transformation at least in a fraction of MDS patients.

2.3.2. *FLT3 Gene*

FLT3 gene encodes receptor-type tyrosine kinase that is involved in proliferation and differentiation of hematopoietic precursor cells. An internal tandem duplication of the human FLT3 gene is found as a somatic mutation in 15-20% of AML and 5% of MDS [57]. This abnormality seems to be a late genetic event during the disease course, and patients with FLT3 mutations tend to have a poor prognosis, suggesting that FLT3 tandem duplication is associated with leukemic transformation from antecedent MDS [58].

2.3.3. *P53 Gene*

The p53 gene is a hallmark of tumor suppressor genes and its alterations are involved in various types of human malignancies. Inactivation of the p53 gene in both alleles by mutations or deletions has been shown to predispose the cells to neoplastic transformation. Inactivation of the p53 gene is detected in 5-10% of MDS, and preferentially in clinically advanced stages and in karyotypically unstable cases with MDS, indicating p53 mutations may play a role in leukemic progression of MDS [59].

2.3.4. *AML1 Gene*

The AML1 (Runx1) gene encoding the heterodimeric transcription factor, which binds to DNA through the Runt domain, is frequently involved in chromosomal translocations associated with human leukemias. Heterozygous missense mutations of the AML1 gene is noted to be causative for familial platelet disorder (FPD) with predisposition to AML, in which haploinsufficiency of AML1 causes an autosomal dominant congenital platelet defect and predisposes to the acquisition of additional mutations that cause leukaemia [60]. Missense mutations, mainly in the Runt domain, of the AML1 gene are also identified in approximately 5% of AML [61]. It is notable that AML1 mutations are preferentially detected in AML of the Mo phenotype with a high frequencies of 22% and that most of them are biallelic, since AML1 protein transcriptionally regulates the expression of the myeloperoxidase gene [62]. Although less frequently, the AML1 gene is also a target of mutations in MDS, and at least some of them show not only a loss-of-function phenotype of AML1 but also a dominant negative effect on normal AML1 function [63].

2.3.5. *C/EBP α Gene*

The transcription factor C/EBP α (for CCAAT/enhancer binding protein- α) is crucial for the differentiation of granulocytes. Conditional expression of C/EBP α triggers neutrophilic differentiation, and no mature granulocytes are observed in C/EBP α -mutant mice. Heterozygous mutations in C/EBP α are found in 7-8% of AML and rarely in MDS [64,65].

2.4. *Epigenetic Alteration*

The cyclin-dependent kinase inhibitor (CDKI) genes, p15INK4B and p16INK4A, are frequently inactivated by genetic alterations in many malignant tumors and are shown to be tumor-suppressor genes [66,67]. p15INK4B gene is an inhibitor of CDK4 and CDK6 whose expression is induced by transforming growth factor (TGF) β [68]. Although genetic alterations in these genes may be limited to lymphoid malignancies [69], it has been reported that their inactivation by aberrant methylation of 5' CpG islands is involved in various hematologic mali-

gnancies [70]. Recent reports suggest frequent methylation of the p15INK4B gene promoter in leukemia cells, and it has been proposed that this methylation could be necessary for leukemic cells to escape TGF β regulation. p15INK4B methylation is observed in 30-50% of MDS cases, and is correlated with the percentage of bone marrow blasts, the risk of disease evolution toward AML, and the poor prognosis, suggesting that p15INK4B methylation play an important role in the pathogenesis of high-risk MDS and is related to leukemic transformation of MDS [71,72]. These suggest that proliferation of leukemic cells might require an escape of regulation of the G1 phase of the cell cycle, and possibly of TGF β inhibitory effect.

Decitabine (5-aza-2'-deoxycytidine) acts as a powerful demethylating agent in vitro. Clinically, low-dose decitabine ameliorates cytopenias including induction of trilineage responses in approximately 50% of patients with high-risk MDS [73]. It is reported that repeated courses of low-dose decitabine induce cytogenetic remissions in a substantial number of elderly MDS patients with pre-existing chromosomal abnormalities; these are associated with improved survival compared with patients in whom the cytogenetically abnormal clone persists [74]. MDS patients with high-risk chromosomal abnormalities may particularly benefit from this treatment.

3. Molecular Pathogenesis

In spite of a multiplicity of endeavor to elucidate the molecular mechanisms of MDS, little is known about the pathogenesis of the first trigger or the early stage of MDS. Based on the accumulated knowledge on the genes involved in chromosomal translocations and somatic mutations, several signal transduction pathways emerge to be focused. Over the last decade, major signal transduction pathways triggered by exogenous stimuli including growth factors were identified and characterized. In relation to oncogenesis, one of the notable signal transduction pathways is now known as the RAS signaling pathway since RAS oncogene product is a key molecule in the development of a wide variety of tumors [75]. Genetic alterations of some molecules on the RAS signaling pathway are revealed to be responsible for pathogenesis of MDS as well as leukemias. They are divided into two categories, receptor tyrosine kinases (RTKs) including FLT3, FMS and KIT, and RAS and GAP-related protein, NF1, which are downstream of RTKs.

Normal cell growth through the cell cycle is regulated by the sequential formation, activation, and subsequent inactivation of a series of cyclin cyclin-dependent kinase (CDK) complexes. The mechanisms underlying the expression of cyclins and the activation of the different cyclin-CDK complexes required for progression through the successive cell cycle transitions are now well understood. In addition to positive regulation by the activation of cyclin-CDK complexes, negative regulation is also required at several checkpoints of the cell cycle. It is now well known that tumors can be caused by

loss of the normal brakes, tumor suppressor genes. p53 stimulates production of p21 which blocks cyclin-CDK complexes and therefore causes G1 arrest [76]. Similarly, p15INK4B, p16INK4A, and RB form complexes with cyclin or CDK, and contribute to cell growth suppression. Among the cell cycle regulators, p15INK4B is most frequently, and p53 is less frequently, involved in the pathogenesis of MDS though not in the early stage but in the late stage of the disease.

In terms of the pathogenesis of the initial development of MDS, the most highlighted molecules are nuclear proteins, especially transcription factors that play important roles both in cellular development and regulation of cell lineage-specific gene expression. Among the genes encoding transcriptional regulators, of which alterations are detected in MDS or MDS/AML, are AML1, C/EBP α , TEL (ETV6), MLL and EVI-1. AML1, C/EBP α , and TEL (ETV6) are demonstrated to be essential in the hematopoietic cell development or differentiation by the gene targeting method in mice. AML1-binding sites were identified in the upstream of genes encoding factors and receptors that determine the lineage specificity of hematopoietic cells. AML1-targeted mice showed embryonic lethality at midgestation due to hemorrhage in the central nervous system and complete block of definitive hematopoiesis [77,78]. C/EBP α is an important mediator of granulocyte differentiation and regulates the expression of multiple granulocyte-specific genes including the granulocyte-colony-stimulating factor (G-CSF) receptor, neutrophil elastase, and myeloperoxidase. Indeed C/EBP α knockout mice display a profound block in granulocyte differentiation [79]. TEL-disrupted mice are embryonic lethal because of a yolk sac angiogenic defect. In the mouse chimeras with TEL-disrupted ES cells, TEL function is essential for the establishment of hematopoiesis of all lineages in the bone marrow, suggesting a critical role for TEL in the normal transition of hematopoietic activity from fetal liver to bone marrow [80].

It should be noted that quantitative or qualitative aberrations of these transcription factors are detected in primary MDS as well as MDS/AML. Since such transcription factors elaborately regulate expression of cell lineage-specific genes that are required for hematopoietic cell differentiation, it is conceivable that their abnormalities could induce unbalance or block of hematopoietic cell differentiation, which might be observed as dyshematopoiesis or ineffective hematopoiesis in MDS. To further demonstrate the early genetic events in development of MDS, it is needed to generate MDS model mice genetically modified with such genetic lesions.

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