Cancer Science
Japanese Cancer Association JCA
Open Access

Review Article

Recent advances in myelodysplastic syndromes: Molecular pathogenesis and its implications for targeted therapies

Hironori Harada and Yuka Harada

Department of Hematology, Juntendo University School of Medicine, Tokyo, Japan

Key words

DNA methylation, gene mutations, myelodysplastic syndromes, RNA splicing, RUNX1

Correspondence

Hironori Harada, Department of Hematology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.
Tel: +81-3-5802-1069; Fax: +81-3-3813-0841;
E-mail: hharada@juntendo.ac.jp

Funding Information

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Received December 2, 2014; Revised January 12, 2015; Accepted January 13, 2015

Cancer Sci **106** (2015) 329–336

doi: 10.1111/cas.12614

Myelodysplastic syndromes (MDS) are defined as stem cell disorders caused by various gene abnormalities. Recent analysis using next-generation sequencing has provided great advances in identifying relationships between gene mutations and clinical phenotypes of MDS. Gene mutations affecting RNA splicing machinery, DNA methylation, histone modifications, transcription factors, signal transduction proteins and components of the cohesion complex participate in the pathogenesis and progression of MDS. Mutations in RNA splicing and DNA methylation occur early and are considered "founding mutations", whereas others that occur later are regarded as "subclonal mutations". *RUNX1* mutations are more likely to subclonal; however, they apparently play a pivotal role in familial MDS. These genetic findings may lead to future therapies for MDS.

Myelodysplastic syndromes (MDS) are a heterogeneous group of refractory hematological malignancies characterized by peripheral blood cytopenias due to ineffective hematopoiesis and risk for progression to acute myeloid leukemia (AML). Myelodysplastic syndromes have been presumed to be stem cell disorders, and it has been suspected that the development of MDS results from accumulation of multiple gene abnormalities, similar to solid cancers. In MDS, gene mutations, rather than karyotype abnormalities, are the major genetic abnormalities. Recent large-scale analysis using next-generation sequencing has greatly improved our understanding of the molecular pathogenesis, biological implications, clinical effects, subclassifications and prognostication of gene mutations associated with MDS.[1–4] Elucidation of associations between gene mutations and clinical phenotypes has advanced rapidly, whereas the molecular pathogenesis based on the function of each variant has not been fully clarified yet. Among the gene mutations identified to date, we have attempted to clarify that *RUNX1* mutations are a pivotal player in the molecular pathogenesis of MDS. It is becoming increasingly clear that *RUNX1* mutations, cooperating with other various gene abnormalities, may promote the development of MDS /AML.

In this review, we initially summarize the various gene mutations that play an important role in the development of MDS. Next, we focus on *RUNX1* mutations, which are key players in familial MDS and are a subject of active research. Finally, we attempt to propose comprehensive molecular mechanisms to achieve a better understanding of MDS, including implications for novel targeted therapies.

Gene Mutations

It has been reported that gene mutations involved in RNA splicing machinery, DNA methylation, histone modifications, transcription factors, signal transduction proteins and components of the cohesion complex participate in the pathogenesis and progression of MDS[5,6]. Recent large-scale analysis using next-generation sequencing has gradually clarified the roles of various gene mutations in MDS. The median number of gene mutations affecting amino acid sequences detected in MDS is approximately 10 per patient.[2–4] However, most of these are randomly acquired non-pathogenic mutations (i.e. passenger mutations), while the recurrent mutations that are causally related to the pathogenesis of MDS (i.e. driver mutations) are limited.[2] Myelodysplastic syndromes are

characterized by such driver mutations in about 40 genes, and approximately 80% of patients have one or more driver mutations.[3,4] The most common genes with driver mutations are listed in Table 1.

Mutations of RNA splicing machinery. To generate a mature mRNA, an RNA splicing process that removes introns from pre-mRNA, mediated by a protein complex called a spliceosome, is essential.[6] Splicing factor mutations, mainly *SF3B1*, *SRSF2*, *U2AF1* (*U2AF35*), and *ZRSR2*, are detected with high frequency in MDS (45–85%), and they occur in a mutually exclusive manner.[7] Because they are rare (<10%) in AML or myeloproliferative neoplasms,[8] we can regard them as MDS-specific mutations. The mutations of these genes seem to have different impacts on cellular functions, manifesting as unique MDS phenotypes and suggesting that MDS phenotypes may be driven by alterations in splicing machinery.[6] Functional studies to elucidate the exact mechanistic significance of mutant proteins and their downstream targets are ongoing.

Mutations in *SF3B1* are detected at an extremely high frequency (60–80%) in refractory anemia with ring sideroblasts,[7,9–11] and the *SF3B1* mutation status has a positive predictive value for the MDS phenotype with ring sideroblasts of 98%.[12,13] Heterozygous mutations are clustered at amino acids 622–700, especially K700E.[8] It is possible that mutations may affect splicing of transcripts coding for proteins associated with iron handling in erythroid precursors, leading to abnormal localization of ferritin in mitochondria, as one of the target genes is *SLC25A37*.[14]

The *SRSF2* gene is also frequently mutated in MDS and more commonly in chronic myelomonocytic leukemia (CMML).[7,15–17] Heterozygous mutations occur almost exclusively at position P95,[8] which is essential for forming extensive contact with the target RNA through stacking.[18] *SRSF2*

Table 1. Summary of driver mutations in myelodysplastic syndromes (MDS)

Mutated genes	Associated phenotypes	MDS types	Other disease	Frequency in MDS (%)	Effect on outcome	Application to treatment
RNA splicing (mutually exclusive)				60–70		None
SF3B1	Ring sideroblasts	RARS, RCMD-RS	RARS-T	15–30	Good	
SRSF2		RCMD, RAEB	CMML	10–20	Poor	
U2AF1		RCMD, RAEB	CMML	5–10	Poor	
ZRSF2		RCMD, RAEB	CMML	5–10	None	
DNA methylation (*TET2* and *IDH1/2* are exclusive)				40–50		DNA methyltransferase inhibitors
TET2	Myeloid dominancy	All MDS, normal karyotype	CMML	20–30	None	IDH1/2 inhibitors
IDH1/2		RCMD, RAEB	CMML	5	Poor (*IDH2*)	
DNMT3A		All MDS	AML	10	None	
Chromatin modification				20–30		Deacetylase inhibitors
ASXL1		RCMD, RAEB	CMML	15–20	Poor	
EZH2	-7/7q-	RCMD, RAEB	CMML	5	Poor	
BCOR		RCMD, RAEB		5	Poor	
Transcriptional factor				20–30		None
RUNX1	Thrombocytopenia	RCMD, RAEB	CMML, AML	10	Very poor	
CEBPA		RCMD, RAEB	AML	<5	None–poor	
ETV6		RCMD, RAEB		<5	Poor	
Signal transduction (mutually exclusive)				20–30		Kinase inhibitors
NRAS/KRAS		All MDS	JMML, CMML	10	Poor	
CBL		All MDS	JMML, CMML	5	Poor	
JAK2	Megakaryocytosis	All MDS	RARS-T, MPN	5	None	JAK inhibitors
NF1		All MDS	JMML	<5	Poor	
FLT3		All MDS	AML	<5	Poor	FLT3 inhibitors
Cohesin complex (mutually exclusive)				10		None
STAG2		RCMD, RAEB	AML, CMML	5–10	None–poor	
TP53	Complex karyotype	RAEB, isolated del(5q)		10	Very poor	None

AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; JMML, juvenile monomyelocytic leukemia; MPN, myeloproliferative neoplasms; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ring sideroblasts; RARS-T, refractory anemia with ring sideroblasts with thrombocytosis; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, refractory cytopenia with multilineage dysplasia with ring sideroblasts.

mutations are associated with mutations of *RUNX1*, *IDH2*, *ASXL1*, *TET2*, and *STAG2*.[3,7,16] Co-mutation of *TET2* and *SRSF2* was highly predictive of a myeloid neoplasm characterized by myelodysplasia and monocytosis, including but not limited to CMML.[13]

Mutations of *U2AF1* (*U2AF35*) are detected in 5–12% of MDS.[7,8,19] *U2AF1* mutations are associated with *ASXL1* mutations.[4] Mutations in *U2AF1* recurrently alter two amino acid residues, S34 in the zinc finger 1 domain or Q157 in the zinc finger 2 domain.[8] U2AF1 mutants induce global abnormalities of RNA splicing, resulting in increased transcripts with unspliced intronic sequences and suppressed cellular proliferation,[7] or they promote enhanced splicing and exon skipping.[19]

Mutations in *ZRSF2* are also detected in 3–11% of MDS. The mutations are widely distributed along the entire protein without a dominant location.[7]

DNA methylation. In MDS, gene mutations involved in DNA methylation are frequently detected, resulting in aberrantly hypermethylated promoter-associated CpG islands. *TET2* and *IDH1/2* mutations occur in a mutually exclusive manner. TET2 is a critical regulator of hematopoietic stem cell (HSC) homeostasis,[20] and TET2 mutants induce self-renewal and clonal expansion in hematopoietic stem/progenitor cells in both normal and malignant hematopoiesis. Loss-of-function mutations or deletion of the gene leads to impaired DNA demethylation. Somatic *TET2* mutations are found in elderly individuals with clonal hematopoiesis without hematological malignancies,[21] suggesting that the mutation is an aging-associated factor of hematopoietic cells or an initiating mutation. IDH1/2 mutants inhibit TET2 function, resulting in a similar effect of TET2 mutants.[22]

The DNA methyltransferase DNMT3A is expressed at high levels in HSCs.[23] *DNMT3A* mutations are frequently detected in AML, and less frequently in MDS.[24] In a mouse model, *Dnmt3a*-null HSCs showed DNA methylation changes and upregulated HSC multipotency genes, including *Runx1* and *Gata3*, resulting in stem cell expansion and reduced differentiation.[23]

Chromatin modification. Gene mutations associated with post-translational modifications of histones are also detected in MDS. ASXL1 plays critical roles both in activation and suppression of Hox genes in axial patterning through regulating the polycomb group and trithorax group proteins. *ASXL1* is mutated in patients with the entire spectrum of myeloid malignancies. *Asxl1*-mutated mice displayed multilineage myelodysplasia, pancytopenia, and occasional progression to overt leukemia, which is similar to human MDS.[25] This phenotype is associated with derepression of *Hoxa9* and microRNA-125a.

EZH2 is a component of the polycomb repressive complex-2 and encodes a histone methyltransferase that initiates trimethylation of lysine 27 in histone 3 (H3K27me3). Somatic loss-of-function mutations of *EZH2* were identified in MDS.[26,27] EZH2 loss also occurs in MDS by -7/7q- chromosome anomalies and reduced expression of EZH2 in $CD34^+$ cells.[28] *EZH2* mutations in lower-risk MDS are correlated with a significantly worse prognosis.[29]

BCOR/BCORL1 is also a polycomb complex component and *BCOR/BCORL1* mutations are detected in MDS associated with an unfavorable outcome.[3,4,30]

Transcriptional factors. Gene mutations of transcriptional factors associated with differentiation of HSCs are also detected in MDS, and they contribute to the differentiation impairment of hematopoietic cells. Furthermore, germ-line mutations of these genes have been described in familial MDS/AML.[31]

The *RUNX1/AML1* gene has been investigated in the pathogenesis of hematopoietic diseases, and *RUNX1* mutations have been frequently detected in patients with various types of hematological malignancies. A heterozygous germline mutation of the *RUNX1* gene is known to cause a familial platelet disorder with a predisposition to AML (FPD/AML), which is regarded as familial MDS.[31] *RUNX1* mutations are detected in 10–20% of MDS,[1] and they are especially frequent in higher-risk MDS and therapy-related myeloid neoplasms,[32,33] where they are associated with severe thrombocytopenia and very poor outcomes.[1,3,4] *RUNX1* mutations have been shown to play a pivotal role in the pathogenesis of MDS/AML in both mouse models and human $CD34^+$ cells.[34,35]

The transcription factor ETV6 is required for HSC maintenance. *ETV6* mutations and deletions are detected in <5% of MDS patients.[1,4] *ETV6* mutations are one of the predictors of poor overall survival in MDS.[1] Other mutations of transcriptional factors, including *CEBPA*, *NPM1*, and *GATA2*, have been described not only in AML but also in <5% of MDS. Most AML patients with *CEBPA* mutations have two different types of mutations simultaneously and show a favorable outcome, whereas MDS patients have only single *CEBPA* mutations with a variety of associated gene alterations and show a poor prognosis.[36]

Signal transduction. Gene mutations involved in the signal transduction pathways occur in a mutually exclusive manner in individual patients with MDS.[1] One of the mutations in this category induces hypersensitive proliferation of MDS stem cells, contributing to the development of MDS. Genes involved in this category are more frequently involved in the pathogenesis of MDS/myeloproliferative neoplasms, especially juvenile monomyelocytic leukemia.

Genes involved in the RAS signaling pathway are mutated in hematopoietic malignancies. In MDS, *NRAS* or *KRAS* mutations are present in approximately 10% of patients, and these typically occur during transformation to AML. Mutations of receptor tyrosine kinases (*FLT3* and *KIT*), commonly seen in *de novo* AML, are infrequent in MDS. Other members of the RAS pathway (*NF1*, *BRAF*, and *PTPN11*) are rarely mutated. CBL is involved in negative modulation of tyrosine kinase signaling. *CBL* mutations are also found in 5% of MDS, resulting in aberrant tyrosine kinase signaling. Activation of the JAK/STAT signaling pathway also associates with MDS pathogenesis. *JAK2* mutations found in 5% of MDS contribute to megakaryocyte proliferation.[13]

Other pathways. The cohesin complex is involved in cohesion of sister chromatids, post-replicative DNA repair, and transcriptional regulation. Gene mutations and deletions of the cohesin complex, including *STAG2*, *RAD21*, *SMC1A*, and *SMC3*, are mutually exclusive and occur in different myeloid neoplasms.[37] *STAG2* mutations are detected in 10% of MDS and result in loss of function. The mutations are significantly associated with *RUNX1* mutations and are most prevalent in higher-risk MDS.[3,38]

TP53 is a tumor suppressor that responds to cellular stress by activating various protective pathways such as cell cycle regulation, apoptosis, and DNA repair.[6] *TP53* mutations occur in approximately 10% of MDS, and they are more frequent in higher-risk or therapy-related cases. The mutations are strongly associated with a complex karyotype, resulting in loss of TP53 function.[1] A subpopulation of isolated del(5q) MDS patients has *TP53* mutations at the early stage of the disease, which is

associated with an increased risk of leukemic evolution.[39] Furthermore, *TP53* mutations are associated with the worst prognosis among the gene mutations in MDS, even after adjustment for other prognostic variables.[1]

Clonal Evolution and Genetic Abnormalities in MDS

Although MDS have been presumed to be a stem cell disorder, chromosomal aberrations were found to be restricted to committed myeloid progenitor cells in MDS.[5] Recently, the clonality and stemness of MDS have been clarified. Nearly all of the bone marrow cells are clonally derived in patients with both MDS and AML developing from MDS, regardless of the myeloblast count.[2] Furthermore, the existence of rare multipotent MDS stem cells was established and all cells identified somatically acquired genetic lesions that were backtracked to distinct MDS stem cells.[40] Similarly, in isolated del(5q)-MDS the acquisition of del(5q) preceded diverse recurrent driver mutations.[40] Therefore, driver mutations occurring in MDS definitely occur in cells with a stem cell phenotype, proving that MDS are a stem cell disease.[41]

By targeted deep sequencing techniques, it has become possible to find driver mutations involved in clonal evolution of MDS.[3,4,42] The mean number of gene mutations in a patient with MDS tends to be higher in the higher-risk subtypes, supporting the idea that some MDS stem cells gain the ability to proliferate by accumulation of gene mutations, leading to clonal expansion and disease progression.[4] Using pairwise precedences comparing the frequencies of mutated alleles, MDS genes were ranked in an attempt to elucidate how early in disease evolution they were mutated. The results suggested that mutations in genes involved in RNA splicing and DNA methylation occur early, that is, they are "founding mutations", whereas driver mutations in genes involved in chromatin modification and signaling often occur later, that is, they are "subclonal mutations".[3,5] Figure 1 shows the relations among founding mutations and subclonal mutations in a clonal evolution from MDS to subsequent AML.[42] Furthermore, combinations that do or do not significantly coexist have been found for each gene mutation, suggesting that the type of founding mutations tends to prescribe the type of subclone mutations.[3,4] In particular, gene mutations involved in RNA splicing are supposed to decide disease types and clinical phenotypes of MDS.[3]

TET2 mutations are also detected in the blood of normal elderly individuals without hematological malignancies,[21] and *DNMT3A* mutations are found in pre-leukemic HSCs in AML patients.[43] Furthermore, MDS-associated gene mutations including *DNMT3A*, *TET2*, *JAK2*, *ASXL1*, *TP53*, *BCORL1*, and *SF3B1* have been recurrently detected in the peripheral blood of patients with cancer without apparent hematological malignancies, the majority being associated with advanced age.[44] The frequency of the gene mutations, which result in clonal expansion, reaches 5–6% of people older than 70 years. These findings support the idea that MDS is an aging disease, and it raises a possibility that anticancer therapies for elderly patients may easily induce therapy-related MDS.

Correlation between Phenotype and Genotype

Myelodysplastic syndromes are distinguished from AML by the blast threshold, defined as 20% blasts in the blood or bone marrow, according to the WHO classification. Unlike the classification for AML, which is based on cytogenetic and genetic abnormalities, the classification for MDS, which is derived from unsatisfactory insights into the molecular pathogenesis, still relies on morphological findings except in the case of isolated del(5q). It will be necessary to clarify the molecular mechanisms of MDS in order to establish a new classification scheme with a characteristic constellation of clinical, genetic, and pathologic findings, similar to AML. Recent large-scale analyses of gene mutations in MDS and related diseases have identified molecular abnormalities (genotypes) that are specific for each MDS subtype (phenotype). *SF3B1* mutations have a strong positive predictive value for the ring sideroblasts phenotype.[7,12,13] According to recent analyses, a classification based on morphologic and genetic criteria for MDS with no excess blasts (<5%) has been proposed.[13] Besides "isolated

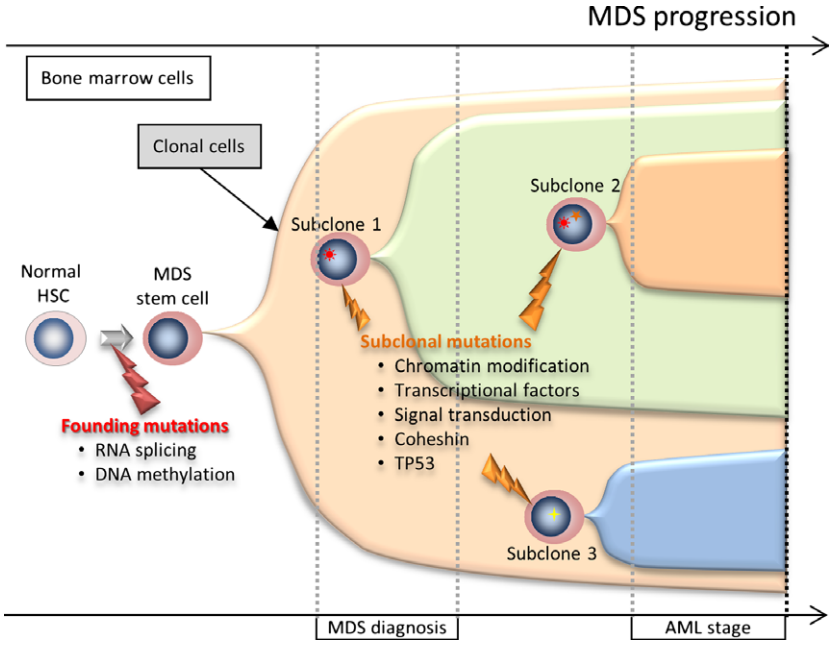

Fig. 1. Clonal architecture of myelodysplastic syndromes (MDS) and progression to acute myeloid leukemia (AML). HSC, hematopoietic stem cell.

del(5q)", which has already been established as a disease category, MDS with "*SF3B1* mutations" may be authorized as a subtype of MDS, corresponding to disease categories in the WHO classification of "refractory anemia with ring sideroblasts (RARS)" and "refractory cytopenia with multilineage dysplasia with ring sideroblasts (RCMD-RS)". Moreover, mutations of genes involved in DNA methylation, the RAS pathway, and cohesion complex and splicing factors except *SF3B1* are independently associated with multilineage dysplasia, and they are regarded as "multilineage dysplasia-associated mutations". Myelodysplastic syndromes with "multilineage dysplasia-associated mutations" form a distinct subset of MDS corresponding to disease categories in the WHO classification of "refractory cytopenia with multilineage dysplasia (RCMD)".[13] Hypocellular MDS without excess blasts is difficult to differentiate from a diagnosis of aplastic anemia. Acquired mutations of myeloid-related genes (*ASXL1*, *DNMT3*, *BCOR*, and *TET2*) are present in a proportion of aplastic anemia and these somatic mutations predict a higher risk of transformation to MDS.[45]

On the other hand, the refractory anemia with excess blasts (RAEB) subtype has not yet been clearly defined by gene mutations. Most patients with MDS-RAEB show clonal evolution into AML or CMML. The WHO classification that defines MDS-RAEB and AML is based solely on the percentage of blasts; however, gene abnormalities are similar between MDS-RAEB and AML with a low blast cell count.[32] In particular, splice factor-mutant RAEB and splice factor mutant AML are clinically, cytologically, and molecularly highly similar, suggesting that these two disorders should be considered as related disease entities.[46] Furthermore, co-mutation of *TET2* and *SRSF2* is highly predictive of myelodysplasia and monocytosis, including CMML.[13,17] Therefore, these disease categories could be distinguished by gene abnormalities. Figure 2 shows a new classification scheme based on the molecular pathogenesis of MDS and related diseases.

Familial/Congenital MDS

Most MDS cases are sporadic, and familial or congenital MDS cases are very rare, but they are valuable for investigations of the molecular pathogenesis of MDS.[31] Germline mutations of hematopoiesis-specific transcriptional factors are implicated in familial/congenital MDS. Familial platelet disorder with predisposition to AML (FPD/AML) is a rare autosomal dominant disorder characterized by congenital quantitative and qualitative platelet defects and a propensity to develop MDS or AML at a high incidence (20–50%). A heterozygous germline mutation of the *RUNX1* gene is known to cause FPD/AML. Induced pluripotent stem cells from FPD/AML pedigrees are clearly defective in the emergence of hematopoietic progenitors and differentiation of megakaryocytes.[47] Affected individuals in FPD/AML pedigrees may develop leukemia at various times throughout their lifespan, suggesting that the acquisition of additional mutations is needed to cause leukemia during this long latency period. *CDC25C* mutations are frequently found in FPD/AML and mutated CDC25C disrupts the G_2/M checkpoint and promotes cell cycle progression. *CDC25C* mutations are suspected to define a founding pre-leukemic clone, followed by stepwise acquisition of subclonal mutations that contribute to leukemia progression.[48] In some FPD/AML pedigrees, all of the affected members develop hematological malignancies, suggesting a possibility that some RUNX1 mutants may upregulate other genes through a gain-of-function mechanism leading to leukemogenesis.

Mutations in *GATA2* have been reported in families that show a predisposition to MDS/AML with primary lymphedema (Emberger syndrome),[49] a syndrome of monocytopenia, B-cell and natural killer cell lymphopenia, and mycobacterial, fungal, and viral infections (MonoMAC syndrome),[50–52] or without preceding symptoms.[53] Molecular mechanisms of processes leading to MDS/AML are being investigated in affected individuals with *GATA2* mutations.

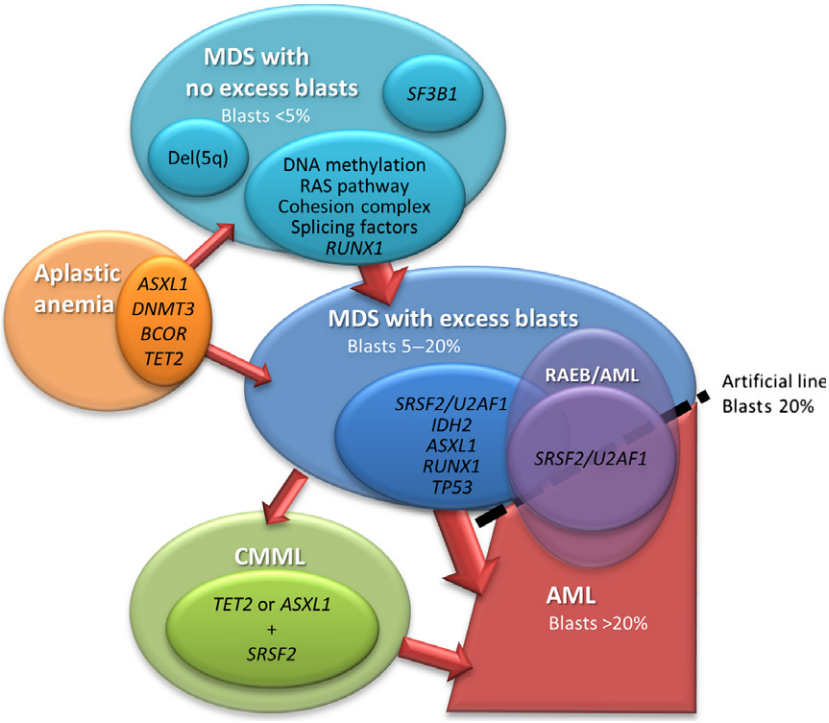

Fig. 2. Myelodysplastic syndromes (MDS) and related hematological diseases caused by driver mutations. AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia.

Molecular Mechanisms of MDS via RUNX1 Abnormalities

Mutations in *RUNX1* are generally subclonal rather than founding mutations in MDS.[3,5] However, in FPD/AML, *RUNX1* mutations apparently play a pivotal role as founding mutations. The *RUNX1* gene has been investigated in the pathogenesis of hematopoietic diseases, and *RUNX1* mutations have been frequently detected in patients with various types of myeloid neoplasms, especially in higher-risk MDS.[35] It is intriguing how *RUNX1* mutations contribute to the development of divergent hematological neoplasms. Functionally, most of the RUNX1 mutants show an equal loss of normal RUNX1 *trans*-activation potential.[54,55] Although a stem/progenitor cell with a *RUNX1* mutation is suspected to have an MDS-genic potential to develop cell dysplasia and self-renewal capacity, it does not have enough proliferation ability for the development of MDS.[35] Therefore, additional gene alterations that induce proliferation activity seem to be necessary. *STAG2*, *EZH2*, and *ASXL1* mutations have significant relationships with *RUNX1* mutations,[3,4] suggesting that these mutations may functionally collaborate with *RUNX1* mutations. The biological functions of RUNX1 mutants in human $CD34^+$ cells and in a mouse bone marrow transplantation model show increased leukemogenic potential with high expression of *Evi1*,[34] overexpression of *BMI1*,[35] or loss of *Ezh2*.[56] Furthermore, alterations of *RUNX1* expression levels and splicing abnormalities, which can be changed by epigenetic modifications, microRNA alterations, and other known/unknown mechanisms, may be associated with the development of hematological malignancies.[6]

Therapeutic Approaches from Molecular Pathogenesis

The current therapeutic algorithm for MDS, dividing cases into lower-risk and higher-risk according to some prognostic scoring systems, is based on the guidelines of the National Comprehensive Cancer Network.[57] Because curative therapies have not been established, patients with MDS without symptoms are carefully monitored without therapy. Lower-risk patients are typically treated with supportive care including transfusion and antimicrobial agents for suspected infections. Iron chelation in erythrocyte transfusion-requiring patients may be considered. Patients with del(5q) are treated with lenalidomide. To stimulate hematopoiesis, erythropoiesis-stimulating agents, granulocyte colony stimulating factor, and thrombopoietin agonists are considered for carefully selected patients.[58] A subset of the patients respond to immunosuppressive therapy. Patients with higher-risk MDS are typically treated with hypomethylating agents, and they can proceed to allogeneic hematopoietic stem cell transplantation, the only potentially curative therapy for MDS, if a donor is available and the patient is a suitable candidate.[57,59] However, most MDS patients exceed the recommended age for safe transplantation. Overall, MDS treatment is disappointing because only some of the patients respond to drug therapy and their responses are often not durable. Genetic studies may provide both insights to better inform treatment choices and new therapeutic options.[57]

Clinical predictors of responses to therapeutic agents have been developed. Isolated del(5q) is the best clinical predictor of responses to lenalidomide to alter treatment decisions.[58] *TET2* mutations and *DNMT3A* mutations can predict responses to hypomethylating agents in MDS patients,[60,61] however their predictive value is insufficient and the mutations do not predict overall survival. The therapeutic agents for MDS still have unknown mechanisms, therefore, it is difficult to predict clinical responses by analyzing gene abnormalities at present. Furthermore, most of the currently available therapies fail within a few years even if initial responses are good. Therefore, new therapeutic agents based on the specific molecular pathogenesis are in great demand. A number of DNA methyltransferase inhibitors, deacetylase inhibitors, and kinase inhibitors are in clinical trials; however, molecular target therapies to normalize RNA splicing, transcriptional regulation, or TP53 function have not yet been established.[57] It is expected that the development of these therapeutic agents will change MDS treatment dramatically in the future.

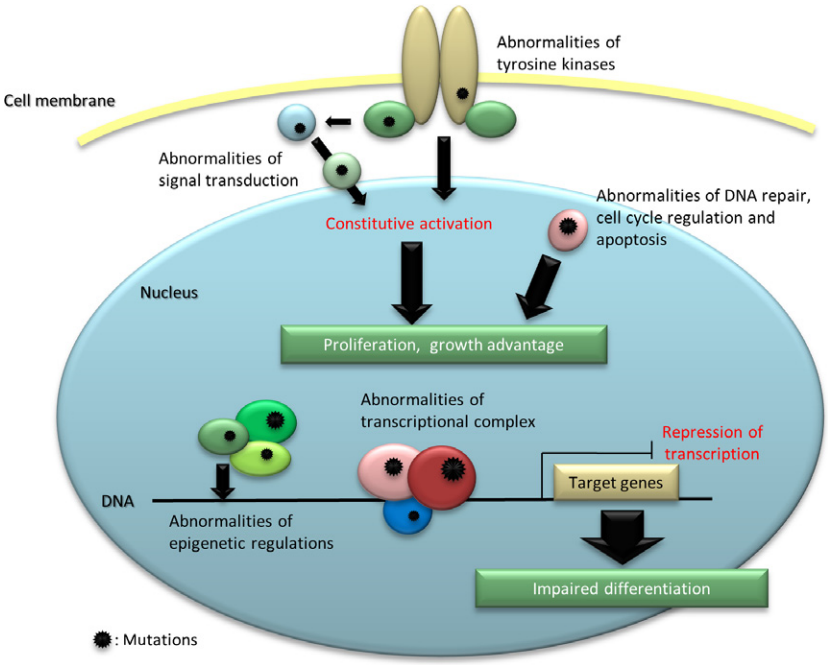

Fig. 3. Molecular pathways and gene mutations associated with myelodysplastic syndromes.

Conclusion

Figure 3 shows molecular pathways and gene mutations affected in MDS. Most of the gene abnormalities in MDS consist of minimum DNA changes with great individual differences. Therefore, diagnosis and classification based on molecular mechanisms cannot yet be readily established. The definition of molecular subclassifications of MDS has advanced gradually: "driver mutations" that lead to pathogenesis have been identified by exhaustive gene mutation analysis, molecular architecture has become clear by deep sequencing, and genotype–phenotype correlations have been clarified by large-scale analyses. However, gene mutation analysis is not yet performed routinely, and its use for diagnosis at the bedside is still far in the future. Establishment of molecular classifications based on pathogenesis, further progress in developing simple methods to detect gene mutations, and discovery of therapeutic agents targeting specific gene abnormalities will lead to the establishment of personalized medicines to treat MDS in the near future.

Disclosure Statement

The authors have no conflict of interest.

References

1 Bejar R, Stevenson K, Abdel-Wahab O *et al.* Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med* 2011; **364**: 2496–506.
2 Walter MJ, Shen D, Ding L *et al.* Clonal architecture of secondary acute myeloid leukemia. *N Engl J Med* 2012; **366**: 1090–8.
3 Papaemmanuil E, Gerstung M, Malcovati L *et al.* Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood* 2013; **122**: 3616–27.
4 Haferlach T, Nagata Y, Grossmann V *et al.* Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia* 2014; **28**: 241–7.
5 Cazzola M, Della Porta MG, Malcovati L. The genetic basis of myelodysplasia and its clinical relevance. *Blood* 2013; **122**: 4021–34.
6 Kulasekararaj AG, Mohamedali AM, Mufti GJ. Recent advances in understanding the molecular pathogenesis of myelodysplastic syndromes. *Br J Haematol* 2013; **162**: 587–605.
7 Yoshida K, Sanada M, Shiraishi Y *et al.* Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature* 2011; **478**: 64–9.
8 Makishima H, Visconte V, Sakaguchi H *et al.* Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. *Blood* 2012; **119**: 3203–10.
9 Malcovati L, Papaemmanuil E, Bowen DT *et al.* Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood* 2011; **118**: 6239–46.
10 Papaemmanuil E, Cazzola M, Boultwood J *et al.* Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N Engl J Med* 2011; **365**: 1384–95.
11 Visconte V, Rogers HJ, Singh J *et al.* SF3B1 haploinsufficiency leads to formation of ring sideroblasts in myelodysplastic syndromes. *Blood* 2012; **120**: 3173–86.
12 Cazzola M, Rossi M, Malcovati L, Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie M. Biologic and clinical significance of somatic mutations of SF3B1 in myeloid and lymphoid neoplasms. *Blood* 2013; **121**: 260–9.
13 Malcovati L, Papaemmanuil E, Ambaglio I *et al.* Driver somatic mutations identify distinct disease entities within myeloid neoplasms with myelodysplasia. *Blood* 2014; **124**: 1513–21.
14 Visconte V, Avishai N, Mahfouz R *et al.* Distinct iron architecture in SF3B1-mutant myelodysplastic syndrome patients is linked to an SLC25A37 splice variant with a retained intron. *Leukemia* 2015; **29**: 188–95.
15 Thol F, Kade S, Schlarmann C *et al.* Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. *Blood* 2012; **119**: 3578–84.
16 Wu SJ, Kuo YY, Hou HA *et al.* The clinical implication of SRSF2 mutation in patients with myelodysplastic syndrome and its stability during disease evolution. *Blood* 2012; **120**: 3106–11.
17 Itzykson R, Kosmider O, Renneville A *et al.* Clonal architecture of chronic myelomonocytic leukemias. *Blood* 2013; **121**: 2186–98.
18 Daubner GM, Clery A, Jayne S, Stevenin J, Allain FH. A syn-anti conformational difference allows SRSF2 to recognize guanines and cytosines equally well. *EMBO J* 2012; **31**: 162–74.
19 Graubert TA, Shen D, Ding L *et al.* Recurrent mutations in the U2AF1 splicing factor in myelodysplastic syndromes. *Nat Genet* 2012; **44**: 53–7.
20 Nakajima H, Kunimoto H. TET2 as an epigenetic master regulator for normal and malignant hematopoiesis. *Cancer Sci* 2014; **105**: 1093–9.
21 Busque L, Patel JP, Figueroa ME *et al.* Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet* 2012; **44**: 1179–81.
22 Figueroa ME, Abdel-Wahab O, Lu C *et al.* Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell* 2010; **18**: 553–67.
23 Challen GA, Sun D, Jeong M *et al.* Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat Genet* 2012; **44**: 23–31.
24 Walter MJ, Ding L, Shen D *et al.* Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. *Leukemia* 2011; **25**: 1153–8.
25 Inoue D, Kitaura J, Togami K *et al.* Myelodysplastic syndromes are induced by histone methylation-altering ASXL1 mutations. *J Clin Invest* 2013; **123**: 4627–40.
26 Ernst T, Chase AJ, Score J *et al.* Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet* 2010; **42**: 722–6.
27 Nikoloski G, Langemeijer SM, Kuiper RP *et al.* Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat Genet* 2010; **42**: 665–7.
28 Jerez A, Sugimoto Y, Makishima H *et al.* Loss of heterozygosity in 7q myeloid disorders: clinical associations and genomic pathogenesis. *Blood* 2012; **119**: 6109–17.
29 Bejar R, Stevenson KE, Caughey BA *et al.* Validation of a prognostic model and the impact of mutations in patients with lower-risk myelodysplastic syndromes. *J Clin Oncol* 2012; **30**: 3376–82.
30 Damm F, Chesnais V, Nagata Y *et al.* BCOR and BCORL1 mutations in myelodysplastic syndromes and related disorders. *Blood* 2013; **122**: 3169–77.
31 Liew E, Owen C. Familial myelodysplastic syndromes: a review of the literature. *Haematologica* 2011; **96**: 1536–42.
32 Harada Y, Harada H. Molecular pathways mediating MDS/AML with focus on AML1/RUNX1 point mutations. *J Cell Physiol* 2009; **220**: 16–20.
33 Harada Y, Harada H. Molecular mechanisms that produce secondary MDS /AML by RUNX1/AML1 point mutations. *J Cell Biochem* 2011; **112**: 425–32.
34 Watanabe-Okochi N, Kitaura J, Ono R *et al.* AML1 mutations induced MDS and MDS/AML in a mouse BMT model. *Blood* 2008; **111**: 4297–308.
35 Harada Y, Inoue D, Ding Y *et al.* RUNX1/AML1 mutant collaborates with BMI1 overexpression in the development of human and murine myelodysplastic syndromes. *Blood* 2013; **121**: 3434–46.
36 Kato N, Kitaura J, Doki N *et al.* Two types of C/EBPalpha mutations play distinct but collaborative roles in leukemogenesis: lessons from clinical data and BMT models. *Blood* 2011; **117**: 221–33.
37 Kon A, Shih LY, Minamino M *et al.* Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. *Nat Genet* 2013; **45**: 1232–7.
38 Thota S, Viny AD, Makishima H *et al.* Genetic alterations of the cohesin complex genes in myeloid malignancies. *Blood* 2014; **124**: 1790–8.
39 Jadersten M, Saft L, Smith A *et al.* TP53 mutations in low-risk myelodysplastic syndromes with del(5q) predict disease progression. *J Clin Oncol* 2011; **29**: 1971–9.
40 Woll PS, Kjallquist U, Chowdhury O *et al.* Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. *Cancer Cell* 2014; **25**: 794–808.
41 Jaiswal S, Ebert BL. MDS is a stem cell disorder after all. *Cancer Cell* 2014; **25**: 713–4.
42 Walter MJ, Shen D, Shao J *et al.* Clonal diversity of recurrently mutated genes in myelodysplastic syndromes. *Leukemia* 2013; **27**: 1275–82.
43 Shlush LI, Zandi S, Mitchell A *et al.* Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* 2014; **506**: 328–33.
44 Xie M, Lu C, Wang J *et al.* Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* 2014; **20**: 1472–8.

45 Kulasekararaj AG, Jiang J, Smith AE *et al.* Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. *Blood* 2014; **124**: 2698–704.
46 Taskesen E, Havermans M, van Lom K *et al.* Two splice-factor mutant leukemia subgroups uncovered at the boundaries of MDS and AML using combined gene expression and DNA-methylation profiling. *Blood* 2014; **123**: 3327–35.
47 Sakurai M, Kunimoto H, Watanabe N *et al.* Impaired hematopoietic differentiation of RUNX1-mutated induced pluripotent stem cells derived from FPD/AML patients. *Leukemia* 2014; **28**: 2344–54.
48 Yoshimi A, Toya T, Kawazu M *et al.* Recurrent CDC25C mutations drive malignant transformation in FPD/AML. *Nat Commun* 2014; **5**: 4770.
49 Ostergaard P, Simpson MA, Connell FC *et al.* Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). *Nat Genet* 2011; **43**: 929–31.
50 Hsu AP, Sampaio EP, Khan J *et al.* Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. *Blood* 2011; **118**: 2653–5.
51 Dickinson RE, Griffin H, Bigley V *et al.* Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. *Blood* 2011; **118**: 2656–8.
52 Pasquet M, Bellanne-Chantelot C, Tavitian S *et al.* High frequency of GATA2 mutations in patients with mild chronic neutropenia evolving to MonoMac syndrome, myelodysplasia, and acute myeloid leukemia. *Blood* 2013; **121**: 822–9.
53 Hahn CN, Chong CE, Carmichael CL *et al.* Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. *Nat Genet* 2011; **43**: 1012–7.
54 Harada H, Harada Y, Tanaka H, Kimura A, Inaba T. Implications of somatic mutations in the AML1 gene in radiation-associated and therapy-related myelodysplastic syndrome/acute myeloid leukemia. *Blood* 2003; **101**: 673–80.
55 Harada H, Harada Y, Niimi H, Kyo T, Kimura A, Inaba T. High incidence of somatic mutations in the AML1/RUNX1 gene in myelodysplastic syndrome and low blast percentage myeloid leukemia with myelodysplasia. *Blood* 2004; **103**: 2316–24.
56 Sashida G, Harada H, Matsui H *et al.* Ezh2 loss promotes development of myelodysplastic syndrome but attenuates its predisposition to leukaemic transformation. *Nat Commun* 2014; **5**: 4177.
57 Bejar R, Steensma DP. Recent developments in myelodysplastic syndromes. *Blood* 2014; **124**: 2793–803.
58 Fenaux P, Adès L. How we treat lower-risk myelodysplastic syndromes. *Blood* 2013; **121**: 4280–6.
59 Sekeres MA, Cutler C. How we treat higher-risk myelodysplastic syndromes. *Blood* 2014; **123**: 829–36.
60 Bejar R, Lord A, Stevenson K *et al.* TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. *Blood* 2014; **124**: 2705–12.
61 Traina F, Visconte V, Elson P *et al.* Impact of molecular mutations on treatment response to DNMT inhibitors in myelodysplasia and related neoplasms. *Leukemia* 2014; **28**: 78–87.