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Pathogenesis of myelodysplastic syndromes: an overview of molecular and non-molecular aspects of the disease

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Abstract

Myelodysplastic syndromes (MDS) are a group of clonal disorders arising from hematopoietic stem cells generally characterized by inefficient hematopoiesis, dysplasia in one or more myeloid cell lineages, and variable degrees of cytopenias. Most MDS patients are diagnosed in their late 60s to early 70s. The estimated incidence of MDS in the United States and in Europe are 4.3 and 1.8 per 100,000 individuals per year, respectively with lower rates reported in some Asian countries and less well estimated in other parts of the world. Evolution to acute myeloid leukemia can occur in 10–15% of MDS patients. Three drugs are currently approved for the treatment of patients with MDS: immunomodulatory agents (lenalidomide), and hypomethylating therapy [HMT (decitabine and 5-azacytidine)]. All patients will eventually lose their response to therapy, and the survival outcome of MDS patients is poor (median survival of 4.5 months) especially for patients who fail (refractory/relapsed) HMT. The only potential curative treatment for MDS is hematopoietic cell transplantation. Genomic/chromosomal instability and various mechanisms contribute to the pathogenesis and prognosis of the disease. High throughput genetic technologies like single nucleotide polymorphism array analysis and next generation sequencing technologies have uncovered novel genetic alterations and increased our knowledge of MDS pathogenesis. We will review various genetic and non-genetic causes that are involved in the pathogenesis of MDS.

Key Words MDS, Molecular mutation, Pathogenesis

INTRODUCTION

1. Overview of myelodysplastic syndromes

Myelodysplastic syndromes (MDS) are clonal stem cell malignancies characterized by cytopenias, inefficient hematopoiesis, dysplasia in one or more myeloid cell lineages and increased risk of development of acute myeloid leukemia (AML). It is sub-classified based on percent of bone marrow (BM) and peripheral blood blasts, type/degree and number of dysplastic cell lineages, presence/absence of ring sideroblasts (RS) and presence of specific chromosomal abnormalities. The median age at the diagnosis of MDS is 71 years [1, 2]. Treatment options for MDS patients vary based on the disease severity. Transfusions, growth factors, and antibiotic therapy are part of the supportive care that is usually suggested for low-risk MDS patients. Chemotherapy regimens like hypomethylating therapy (HMT; decitabine and 5-azacytidine), immunomodulatory agents (lenalidomide), cytarabine, idarubicin, and daunorubicin are commonly used in high-risk MDS patients to delay the AML transformation. Ultimately, long term remission is achieved by using high dose chemotherapy and hematopoietic stem cell (HSC) transplantation.

Genetic defects (chromosomal aberrations, gene mutations, copy-number alterations, abnormal gene expression) are common in MDS. Clonal and recurrent cytogenetic abnormalities and their frequency are summarized in Table 1. They are often present at disease presentation; however abnormal clones can appear during the disease course and are associated with worsening conditions. Genomic instability (deletions, translocations) is important since these genetic events can encompass regions containing tumor suppressor genes (TSGs) relevant to MDS biology. Unbalanced translocations are found in ~50% of primary and 80% of therapy-related MDS (t-MDS) cases [3, 4]. Complex kar-

Table 1. Clonal recurrent cytogenetic abnormalities and their frequency in myelodysplastic syndrome.

Chromosomal abnormalities	Primary MDS (%)	Therapy-related MDS (%)
Unbalanced		
-5 or del(5q)	10	40
-7 or del(7q)	10	50
+8	10	23[b]
del(20q)	5–8	2.2[c]
-Y	5	NA
i(17q) or t(17q)	3–5	Rare[d]
del(11q)	3	3.9[e]
del(12p) or t(12p)	3	NA
-13 or del(13q)	3	1.2
idic(X)(q13)	1–2	NA
del(9q)	1–2	NA
Balanced		
t(11;16)(q23;p13.3)		3.0
t(3;21)(q26.2;q22.1)		2.0
t(1;3)(p36.3;q21.2)		NA
t(2;11)(p21;q23)		NA
inv(3)(q21q26.2)	1	0.2
t(6;9)(p23;q34)	1	0.2
t(11q23)[a]	1	12
t(21q22)[a]	1	2.6
t(15;17)[a]		2.0
inv(16)[a]		2.0
t(8;16)[a]		0.3

[a]This frequency was reported by Smith *et al.* [121]. [b]Data are extrapolated by Koh *et al.* [122]. [c]The frequency was reported in Mauritzson *et al.* [123]. [d]Abnormalities of the chromosome 17 such as 17q (del or t), 17p (del or t), -17 are detectable but rare. [124]. [e]The frequency refers to 11q- in Mauritzson *et al.* [123].
Abbreviations: MDS, myelodysplastic syndrome; del, deletion; t, translocation; I, isochromosome; inv, inversion; idic, isodicentric; NA, not applicable.

yotype (3 or more defects) is usually correlated with dismal outcomes and can be seen following chemo/radiotherapy and toxic chemicals exposure. The recently published Revised International Prognostic Scoring System (R-IPSS) includes more specific cytogenetic prognostic subgroups to improve prognostic stratification in MDS [5].

Somatic mutations in multipotent stem cells are believed to contribute to MDS pathogenesis, even though no specific defect has been clearly identified. Genomic instability (genetic defects, mutations) increases the propensity to develop AML. It is believed that ~78% of MDS patients carry at least one somatic mutation [6]. The importance of recurrent mutations resides in their potential clinical applications specifically in prognosis, diagnosis, risk stratification, and treatment response. For example, *TP53, EZH2, ETV6, RUNX1*, and *ASXL1* mutations are associated with poor overall survival (OS) [7, 8]. *TP53* mutations predict for progression in low-risk MDS [9] and *TET2*, *DNMT3A* and *SF3B1* mutations predict for response to HMT [10]. *SF3B1* mutations have recently been studied as diagnostic biomarkers in the differentiation between clonal and non-clonal causes of MDS [11].

Sanger sequencing, high resolution whole genome scanning technologies [single nucleotide polymorphism arrays (SNP-A) genotyping], and high-throughput next generation sequencing [HT-NGS, whole exome/genome sequencing (WE/WGS), deep sequencing] have brought to light the presence of mutations in genes of methylation, transcription, signaling, histone modification, RNA-splicing and other pathways. Ultimately, understanding the molecular alterations of genes relevant to MDS will hold the key of targeted therapy and improvement in therapeutic outcomes. Fig. 1 shows a schema of the histopathologic, cytogenetic, and molecular genetic tools for diagnosis, classification, and prognosis assessment in MDS.

2. Evolution of technologies in the detection of chromosomal defects and cryptic lesions in the diagnosis of MDS

Chromosomal abnormalities (-5/5q-, -7/7q-, +8, 20q-, +21, 12p-, 13q-, and 17p-) are detected in 40–60% of primary MDS and considered an important determinant in the prognostic scoring systems [12, 13]. However, conventional metaphase cytogenetics (MC) reaches 10% sensitivity and is informative in 46–59% of MDS patients due to limited results in non-viable cells or non-informative karyotypes [14]. Some subtle chromosomal abnormalities can be undetectable or masked [15]. Fluorescence *in situ* hybridization (FISH) for chromosomes 5, 7, 8, 11, 13, and 20 has complemented MC in cases of undetectable defects and no growth cases. Studies performed to compare FISH to MC have not clarified whether FISH can outperform MC [16, 17]. Recently, FISH has been combined with spectral karyotyping to improve the detection of minimal chromosomal rearrangements and monosomies [18].

The low sensitivity of MC has been overcoming using a powerful method called SNP-A karyotyping. SNP-A, which uses DNA hybridization and fluorescence technique, is currently used in clinical centers as a diagnostic tool to improve the detection rate. SNP-A can detect cryptic lesions [copy neutral-loss of heterozygosity (CN-LOH) or acquired somatic uniparental disomy] in 50% of MDS patients with normal karyotype [19-21]. Part of these lesions can be pathogenic. Studies have demonstrated the value of combining MC with SNP-A and the prognostic importance of the number of SNP lesions in MDS [20, 22, 23].

In the latest 10 years, second-generation DNA sequencing such as HT-NGS has been implemented in the discovery of genetic alterations in cancer. These technologies refer to non-Sanger DNA sequencing methods where millions of DNA strands can be massively sequenced [24]. More recently WE/WGS and deep sequencing have been helpful in discovering germ-line and somatic variants in MDS. These technologies are almost being considered as diagnostic tests compared to direct-sequencing. At the transcript level, RNA-sequencing has been used for a variety of studies (gene expression, transcript isoforms, small RNAs, TCRβ/BCR repertoires, exon usage/splicing patterns, and methylation/chromatin changes). These technologies rely on a high resolution, depth of coverage (number of times a nucleotide is se-

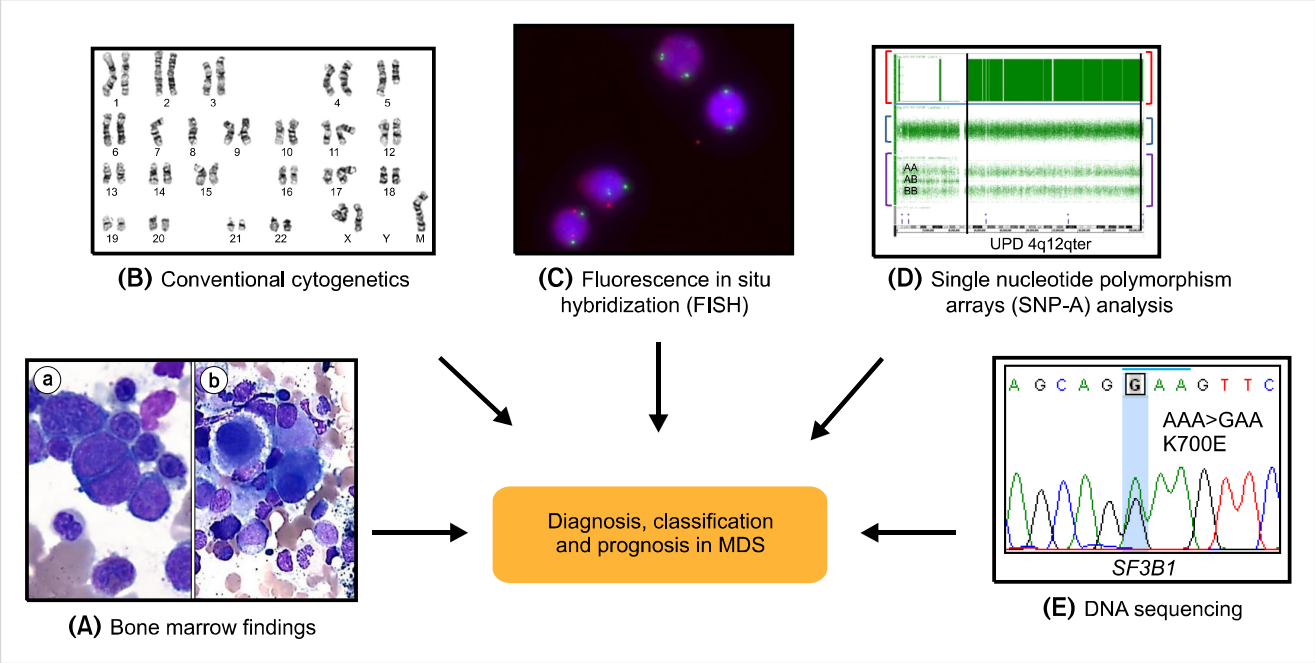

Fig. 1. Diagram of the histopathologic, cytogenetic, and molecular genetic tools for comprehensive evaluation of diagnosis, classification and prognosis in myelodysplastic syndrome. **(A)** Dyserythropoiesis (a) and dysmegakaryopoiesis and dysgranulopoiesis (b) in bone marrow aspirate smears (Wright Giemsa stain ×1,000). **(B)** Conventional cytogenetics showing a 45,XX,del(5)(q22q35),-7,der(17)t(7;17)(p12;p11.2),-8,der(11)t(8;11)(q11.2;p11.2),+mar karyotype. **(C)** Fluorescence in situ hybridization detecting the deletion of the long arm of chromosome 20 with one orange signal using LSI D20S108 probe (target locus on 20q12) and two green signals using CEP 8 probe (target locus on 8p11.1-q11.1) (Abbott Molecular, Abbott Park, IL, USA). **(D)** Representative single nucleotide polymorphism arrays (SNP-A) analysis of loss of heterozygosity (LOH), uniparental disomy (UPD) and gain lesions. The first and top track shows LOH (red brackets), the second track shows copy number for each SNP (blue brackets) and the third track shows the genotype calls (purple brackets). Allele calls are: AA, AB, BB are indicated. Vertical lines indicate each region of the genome. **(E)** Chromatogram of Sanger sequencing showing the forward sequencing of *SF3B1* exon 15 illustrating the most frequent missense mutation (AAA>GAA;K700E; c.2098 G>A).

quenced) and variation/phred score (index of the quality of the variant calls) [25].

MOLECULAR GENETIC ASPECTS IN THE PATHOGENESIS OF MDS

Genetic technologies including HT-NGS have discovered several mutated genes clustered in specific pathways. We will discuss the frequency, function, and prognostic significance of the main pathways and genetic alterations in MDS (Table 2).

1. Epigenetic pathway: *DNMT3A, TET2, IDH1/2, ASXL1, EZH2, UTX*

Epigenetic regulation is one of the main mechanisms of controlling gene function. DNA methylation and histone modification are the 2 epigenetic processes that have been found to be altered in MDS. Aberrant methylation of TSG promoters is present in MDS [26]. Indeed DNA methylation (addition of a methyl-group to DNA) occurs at CpG sites (regions in which a cytosine and a guanine are linked by a phosphate). Since the CpG dinucleotides are localized in upstream regulatory regions, the methylation of the CpG leads to a silencing mechanism. DNA methyltransferases (DNMTs) such as DNMTs 1, 3a, and 3b are enzymes responsible for DNA methylation and highly expressed in AML and other myeloid neoplasms.

A somatic frameshift mutation in *DNMT3A* was first identified in an AML patient by WES. *DNMT3A* mutations were found to be recurrent in 4.1–22.1% of AML patients [27]. The most frequent mutation at position R882 was associated with dismal prognosis and reduced *DNMT3A* activity [28, 29]. The NH2-terminus of *DNMT3A* contains motifs important for the recruitment of transcriptional repressors. *DNMT3A* is a DNA-regulator acting as a check-point of DNA replication by keeping *de novo* methylation regions partially methylated. *DNMT3A* has been found mutated in 8% of MDS patients [30, 31] and 23.4% (23/98) of t-MDS and secondary AML (sAML) patients [32]. Moreover, heterozygous mutations occur in 3.3% of high-risk MDS. *DNMT3A* mutations are highly expressed in the mutated specimens regardless of blast counts, downregulate HSC differentiation, and are associated with worse prognosis and rapid progression to AML [33, 34]. The importance of *DNMT3A* mutations resides in the fact that HMT is considered one of the primary treatments in MDS and acts by altering the aberrant methylation through inhibition of DNMTs. We first reported that the presence of *DNMT3A* and *TET2* mutations confer a better response to DNMT-inhibitors [10]. Itzykson *et al.* [35] showed that high-risk MDS patients carrying *TET2* mutations have better response to 5-azacytidine.

TET2 mutation was found in 20–25% of MDS patients

Table 2. Frequency and prognostic significance of somatic molecular mutations in myelodysplastic syndrome.

Gene	Locus (Entrez Gene cytogenetic band)	Frequency (%)	Prognostic significance
Epigenetic pathway			
DNMT3A	2p23	8	Adverse[a]
TET2	4q24	20–25	Unclear
IDH1/2	2q33.3/15q26.1	1–5	Unclear
ASXL1	20q11	21	Adverse
EZH2	7q35-q36	2	Adverse
RNA splicing machinery			
UTX	Xp11.2	1–2	NA[c]
SF3B1	2q33.1	60[b]	Good
U2AF1	21q22.3	6–12	Unclear
SRSF2	17q25.1	6–12	Poor
ZRSR2	Xp22.1	3.1	NA[c]
PRPF8	17p13.3	3.3	Unclear
Signaling pathway			
JAK2	9p24	6.2–8.3	NA[c]
CBL	11q23.3	1–2	NA[c]
Transcriptional factors and corepressors			
TP53	17p13.1	8	Adverse
RUNX1	21q22.3	9	Adverse
BCOR/BCORL1	Xp11.4/Xq25-q26.1	6–9.1	Adverse
RAS family pathway			
N/K-RAS	1p13.2/12p12.1	2–6	Adverse
Cohesin family pathway			
STAG2	Xq25	5.9%	Adverse[d]
RAD21	8p24	2%	Adverse[d]
SMC1A	Xp11.22-p11.21	<1%	Adverse[d]
SMC3	10q25	2%	Adverse[d]
Less frequent molecular mutations			
SETBP1	18q21.1	2.2	Adverse

[a]An adverse prognostic impact [30]. [b]Indicates the frequency in refractory anemia with ring sideroblasts. [c]The prognostic impact of mutations in these genes cannot be statistically assessed due to the low frequency of mutations. [d]A poor overall survival was associated mainly with *STAG2* mutations in MDS patients [102].

[36, 37] and usually associated with advanced age and normal karyotype. There was no difference in terms of frequency among IPSS categories (24.5% in low/intermediate-1 and 20% in intermediate-2/high-risk MDS). Mutations are present at diagnosis and appear to be stable during disease progression. *TET2* is a dioxygenase catalyzing the conversion of the 5-methylcytosine (5-mc) to 5-hydroxymethylcytosine (5-hmc) by oxidation. Indeed, methylation at the C5 position of cytosine appears to be an epigenetic modification which plays an important role in transcriptional regulation. *TET2* mutations are loss-of-function and correlate with decreased 5-hmc and increased 5-mc levels leading to DNA-hypermethylation and gene silencing [38, 39]. A fraction of wild type (WT) patients also have low 5-hmc. A report correlated the low 5-hmc to the over-expression of the CXXC domain of *TET2* encoding *IDAX* gene [38]. Further, reports have described that low 5-hmc levels are associated with DNA-hypomethylation in myeloid malignancies and DNA-hypermethylation in AML [40]. *TET2* deletions lead to increased HSC proliferation and myeloid differentiation. In mice, *TET2* haploinsufficiency leads to increased myeloid proliferation [41]. Recent studies showed that *TET2* might be under the control of >30 microRNAs (miRNA), which inhibit *TET2* activity and decrease 5-hmc. In-vivo expression of miRNAs targeting *TET2* perturbs the hematopoiesis leading to myeloid cells expansion. Conversely co-expression of *TET2* and miRNA restores the normal phenotypes [42]. Although the acquisition of *TET2* mutations appears to be an important event in the pathogenesis and the transformation of MDS, the prognostic significance remains unclear. Some studies showed favorable prognosis, and other studies revealed no prognostic impact in MDS [43, 44].

Mutations in the isocitrate dehydrogenase 1 (*IDH1*) gene were discovered in ~70% of patients with gliomas through genome-wide association studies. WGS identified a somatic mutation (R132C) in an AML case. Mutations are heterozygous (R132 in *IDH1* and R140 or R172 in *IDH2*). These 2 NADP-dependent IDH were discovered to be mutated at low frequency (~5%) in MDS. A study showed *IDH1/2* mutations in 12% of MDS patients (N=277) and higher frequency in *IDH2* (9%) than in *IDH1* (2.9%). A higher frequency was found in high-risk MDS (23%; 8/35 RAEB-2) and 10–20% of sAML patients [45]. Mutations were associated with normal or intermediate-risk karyotype [46].

IDH1 mutations alter the catalytic activity of the enzyme producing the oncometabolite (*R*)-2-hydroxyglutarate (R2HG), which affects the redox state of the cells and blocks NADPH production. R2HG accumulates in AML cells. Studies have postulated that mutations (loss-of-normal allele, dominant-negative effect) might alter the mitochondrial function. Further, *IDH1/2* mutations lead to DNA-hypermethylation [47]. It has been reported that serum R2HG levels can predict clinical outcomes in *IDH1/2* mutant patients [48]. *IDH1/2* mutant and *TET2* loss-of-function cells have similar abnormal hematopoietic differentiation and methylation profiles although mutations in these genes are mutually exclusive. The presence of mutations in genes of the methylation pathway has been associated with better response to HMT [10]. This finding may help in designing novel therapeutic agents targeting specific gene mutations. Recently, primary AML cells carrying *IDH1/2* mutations have been found to be sensitive to DOT1L inhibitors. DOT1L is a histone methyltransferase responsible for trimethylation of the histone 3 on lysine 79 (H3K79) [49].

ASXL1 is a member of the polycomb-group (PcG) proteins and implicated in chromatin remodeling and homeotic gene repression. *ASXL1* protein is an enhancer of the trithorax- and PcG-proteins in Drosophila driving the bidirectional homeotic determination of the segments during the formation of the embryos. Truncation mutations disrupting activating domains (PDH, RARα, and SRC1) were first reported in MDS in 2009 [50]. *ASXL1* monoallelic mutations have been found in 16% of high-risk MDS and ~30% of sAML with a prior MDS. *ASXL1* mutations have been associated with worse OS [7]. The importance of *ASXL1* resides in its genomic location (20q11.21). Indeed deletion of the long arm of chromosome 20 [del(20q)] are common in MDS. In our experience, *ASXL1* mutations were found in 10% of del(20q) patients [51]. A recent meta-analysis performed on 3,311 adult AML patients has confirmed that *ASXL1* mutations cluster in a specific subtype of AML with poor prognosis [52]. In another study, 16.3% (25/153) of del(20q) patients were mutated with a higher frequency in advanced MDS [53]. Mutational screening revealed the co-occurrence of *ASXL1* with *TET2*, *IDH1/2*, and *EZH2* mutations [54]. *ASXL1* protein interacts with other PcG-proteins (*SUZ12*, *EZH2*), which have a role in histone-modification. In mice, *ASXL1* depletion does not lead to impairment in the stem cells but leads to defects in the lymphoid or myeloid progenitors. Furthermore, mice do not develop MDS or leukemia most probably due to the compensation of other ASXL-members [55, 56].

Deletions of chromosome 7/7q are common in MDS and correlate with poor prognosis. *EZH2* gene encodes the histone methyltransferase factor constituting the catalytic unit of the polycomb repressive complex 2 (PRC2) and promotes the repression of TSGs by depositing the histone mark H3K27me3. Point/inactivating mutations were found in 2–6% of MDS patients [57]. EZH2 forms the PRC2 with EED, SUZ12, and RBBP4 proteins. Mutations in other PRC2 genes are uncommon in MDS (<1%). In other myeloid neoplasms, *SUZ12* deletions are concomitant with *EZH2*. Analysis of 288 low-risk MDS patients showed that *EZH2* mutations were enriched in patients with worse prognosis, were associated with a hemoglobin <10 g/dL ($P \leq 0.008$), and significantly predicted for OS after adjusting for low-risk IPSS [Hazard ratio (HR) ≥2.84] [8].

The Jumonji C (JmjC)-domain family of histone demethylases with the function of removing methyl-groups from the histone methylation site has been studied in MDS. Deletions in H3K27me2/3 (UTX/JMJD3), a demethylase on the X chromosome and in other JmjC members have been found at <1% in MDS with highest frequencies in chronic myelomonocytic leukemia (CMML) (8%) [58].

2. RNA-splicing machinery: *SF3B1, U2AF1, SRSF2, ZRSR2, PRPF8*

Somatic mutations in components of the RNA-splicing machinery were discovered using WE/WGS in myeloid and lymphoid disorders [59]. Splicing factor genes are mutated in almost half of the MDS patients and are generally mutually exclusive and disease-type specific. Sometimes, splicing-gene mutations can also occur with other genetic mutations primarily involved in epigenetics as in the case of *SF3B1* with *TET2* and *DNMT3A*, *U2AF1* with *ASXL1* and *DNMT3A*, and *SRSF2* with *TET2* and *IDH1*. Mutations are localized in hotspot regions [*U2AF1* (S34, Q157), *SRSF2* (P95) and *SF3B1* (mainly K700E)]. Absence of homozygous mutations suggests a gain-of-function while occurrence of frameshift and nonsense in *ZRSR2* gene suggests a loss-of-function. *SF3B1* mutations are frequent in low-risk MDS and associated with RS, whereas *U2AF1* and *SRSF2* mutations are associated with high-risk MDS. Prognosis is favorable in *SF3B1* mutants in MDS, while mutations have negative impact in lymphoid disorders [60, 61]. *SF3B1* mutations occur in 68–75% of RARS and 81% of RARS-T patients [62, 63], while are rare in other diseases [64]. RARS/-T patients carrying *SF3B1* mutations had a favorable clinical outcome [63]. Another study has shown no significant OS and AML transformation in 317 MDS patients [65]. *SF3B1* mutants have been shown to respond better to HMT [10], and preliminary data suggest that *SF3B1* mutations confer better response to erythropoiesis-stimulating agents [63].

SF3B1 mutations were associated with RS formation and recently with the higher expression level of a variant of Mitoferrin-1, a mitochondrial iron importer [66] while other studies suggested an association between *SF3B1* and *ABCB7*, a mitochondrial gene linked to RARS pathogenesis. Nikpour *et al.* [67] used knock-down experiments of *SF3B1* in K562 cells and induced a downregulation of ABCB7 suggesting a link between *SF3B1* mutations and the down-regulation of ABCB7. In the first study investigating the relation between *SF3B1* mutations and the RS phenotype in MDS, RS were increased in the BM of *Sf3b1*$^{+/-}$ mice. We first reported a long term follow-up study of *Sf3b1*$^{+/-}$ mice demonstrating macrocytic anemia, extramedullary hematopoiesis, and thrombocytosis. This feature was most probably correlated with the overexpression of JAK2 that has been correlated with thrombocytosis in humans [68, 69]. Subsequent other studies, focusing primarily on BM reconstitution property

of the HSCs and not on the hematologic or BM phenotype, showed a compromised HSC function [70, 71].

ZRSR2 mutations were found at lower frequency in MDS with no association with RS [72] and not frequent in other BM failure syndromes [64]. Thol *et al.* [72] published that 6/193 (3.1%) MDS patients harbored mutations in coding-exons, while 2 patients exhibit mutations in the introns 7 and 8. Survival analysis was not performed due to the limited number of mutant patients, although 3 patients expired at 56 days, 1.4 and 3.5 years from the time of screening.

Spliceosome inhibitors have been proposed to target the mutant allele in splicing factors in MDS. Pladienolide B, Sudemycins, and Spliceostatin A derived from bacterial fermentation products and small molecules (steroids, biflavonoid natural plant, indole derivatives, protein phosphatases and benzothiazole inhibitors) showed antitumoral effect against the spliceosome [73]. *In vitro* data on Meayamycin, an analog of FR901464 binding to the splicing factor 3b complex and inhibiting pre-mRNA-splicing was reported in 2012 [63].

U2AF1 mutations (S34, Q157) were found in 8.7% of primary MDS patients and were associated with AML progression [74, 75]. *U2AF1* mutations result in a gain-of-function and an increased exon skipping was observed when the mutant S34F cDNA was expressed *in vitro* [74]. *U2AF1* mutations have been shown to cause missplicing and differential exon-usage in mitotic genes (*CEP164, EHMT1, WAC, ATR*), RNA-processing (*PTBP1, STRAP, PPWD1, PABPC4, UPF3B*), and G2/M checkpoint genes (*CEP164*) [76]. It has been also reported that mutant *U2AF1* murine cells have increased sensitivity to Sudemycin [77].

SRSF2 mutations were found in 12.4% of MDS patients with higher ranges in CMML (28.4–47%) [72]. The most common mutations are missense at P95 position. Rare deletions [P95_R102del (c.284_307del), P95_D97del (c.283_291del)] have been also described [78]. Given that studies in *Srsf2*$^{-/-}$ mice demonstrated increased apoptosis and no hematopoietic cells, *SRSF2* is essential for hematopoiesis during embryonic development and induces alternative splicing of hematopoietic genes. Since MDS patients are usually heterozygous, studies using *Srsf2*$^{+/-}$ mice revealed alternative splicing of hematopoietic genes but no obvious hematopoietic defect [79]. *SRSF2* mutations have been correlated with older age, anemia, and normal karyotype. Some studies have shown no impact on OS while others poor outcomes. *SRSF2* mutant had outcomes similar to WT patients in a cohort that underwent allogeneic HSC transplantation suggesting that transplant is able to compensate for the negative impact [80].

PRPF8 is a component of the catalytic core of the spliceosome and forms interactions with the 3' and 5' splice, the branch point, and the excised introns. Two mutant cases were reported by Makishima *et al.* and Gomez-Segui *et al.* [75, 81, 82]. In a larger cohort (N=447) the authors found mutations in 15/447 (3.3%) patients [83]. Mutations were found along the gene, and D1598 was the most common amino acid change. A higher frequency was found in primary and sAML. In total, 60% of the patients appeared to have RS. Due to the genomic mapping of *PRPF8* (17p13.3) and *TP53* (17p13.1), the authors described that some cases with del(17p) also involved the *TP53* locus. Gene expression of TCGA database found that *PRPF8* may regulate mitochondrial respiratory chain genes (*NDUFAF6, SFXN2, SLC25A19*). Knock-down of *PRPF8* in K562 cell line, whole BM and CD34^{+} cells of healthy subjects leads to increased cell proliferation. Cells with *PRPF8* mutant were more sensitive than normal BM cells to Meayamycin [83]. In a model of zebrafish carrying a premature stop codon, an impaired myeloid differentiation and a decreased expression of myeloid compared to erythroid markers were observed. Further, *PRPF8* seems to be able to auto-regulate his own pre-mRNA-splicing. Indeed *PRPF8* splicing in this zebrafish model is defective [84].

3. Signaling pathway: *JAK2, CBL*

JAK2 is a member of the Janus kinase family (*JAK1, JAK3, TYK2*) proteins, which are intracellular non-receptor tyrosine kinases with a function in cell proliferation and survival of HSC. *JAK2* mutations are hallmark of myeloproliferative neoplasms (MPN). The most common mutation at position 617 (V617F) induces autophosphorylation, gene transcription, and *in vitro* kinase activity. In *JAK2* mutant MPN patients, JAK2 phosphorylates the arginine methyltransferase PRMT5 reducing the methylation to histone substrates and promoting the MPN-like phenotype. CD34^{+} cells of *JAK2* mutant MPN patients with decreased PRMT5 expression showed an increased erythroid differentiation and colonies *in vitro* [85]. A gain-of-function mutation in exon 12 is detected in 3–5% of *JAK2* WT patients. Compared to V617F mutation, mutations in exon 12 produce increased ligand-independent signaling and phosphorylation through JAK2. *JAK2* mutations have been reported at frequency of 6.2% in 97 MDS patients carrying isolated del(5q) [86] and in 8.3% (1/12) where the unique mutant patient carried a del(20q) abnormality [87]. In the first study, mutant tended to have elevated platelet count compared to WT patients (475 vs. 250 × 10^9/L; P=0.15). A higher prevalence of *JAK2*^{V617F} was found in patients with RARS-T. Szpurka *et al.* [88] showed that in a group of 9 RARS-T patients, 6 patients carried *JAK2*^{V617F} mutation and 1 WT patient had increased phospho-STAT5 staining. The features of RARS-T resemble more MDS patients in terms of degree of anemia and survival outcomes. It is believed that RARS patients may acquire *JAK2* mutations during the disease course [89]. On the contrary, a study found *JAK2* mutation only at the time of development of myelofibrosis (MF) in 2/6 MDS patients, but not in MDS patients without MF (N=38) [90].

CBL gene encodes for a cytosolic protein, which downregulates the tyrosine kinase signaling trigged by E3 ubiquitin ligase leading to lysosomal/proteosomal degradation and modulates *JAK2* and *MPL* downstream signals. *CBL* mutations seem to trigger an oncogenic phenotype and cell proliferation by activating the RAS-pathway. In mice *CBL* haploinsufficiency leads to MPN-like features (splenomegaly, cell proliferation, sensitivity to growth factors). Mutations in *c-CBL*, *CBL-b*, and *CBL-c* are rare in MDS (1%) while

are more frequent (8.1%) in MDS/MPN, specifically in CMML (15–20%) [91]. The mutations are missense or in-frame deletions and coexist with *JAK2*, *TP53*, *FLT3*, and *RUNX1* mutations.

4. Transcriptional factors and corepressors: *RUNX1*, *TP53*, *BCOR/BCORL1*

RUNX1 [AML1 or core-binding factor-α (CBFA)] is a DNA core-binding factor regulating the transcription of genes important in HSC formation. Indeed, *RUNX1*$^{-/-}$ mice die in utero without achieving complete hematopoiesis. *RUNX1* mutations have been associated with translocations [t(8;21), t(3;21), t(12;21)]. It has been reported that *RUNX1* mutations are present in 12% (16/132) of MDS patients at diagnosis, and they remain unchanged during the disease course. In this study, 2/45 serially available samples acquired *RUNX1* mutations (one at the time of progression to AML and another at relapse and AML transformation after HSC transplantation) [92]. *RUNX1* mutations are associated with high-risk MDS. Steensma *et al.* [93, 94] reported a frequency of 9.6% (5/52) in MDS and higher frequency in RAEB-2 (4/7; 57%).

TP53 is a TSG that regulates the cell cycle progression and the apoptosis. The coexistence of MDS features like cytopenias and hypercellular BM has been correlated with the increased apoptosis in the HSCs. Mutations in *TP53* were detected early at MDS presentation, suggesting a primary event in the disease manifestation and were associated with certain cytogenetic changes including isolated del(5q), -5/5q-, 17p- and complex karyotypes. A study of 318 MDS patients found a mutational frequency of 9.4% (30/318) with 8.1% in primary MDS patients. *TP53* mutations were correlated with complex karyotype and -5/5q- (72%), IPSS intermediate-2/high, and higher blast count. *TP53* mutant had higher leukemic transformation, shorter median OS and progression free survival (PFS) compared to WT patients [8, 96]. *TP53* was also the most commonly mutated gene in t-MDS (21%; 8/38), where *TP53* mutant carried dismal survival compared to WT patients (8.8 vs. 37.4 months; *P*=0.0035) [95]. Some *TP53* mutant patients were also mutated for other genes like *U2AF1*, *RUNX1*, *ASXL1*, *TET2*, *DNMT3A*, and *IDH1*. *TP53* mutant clones reduced in patients responding to 5-azacytidine [96]. A study showed that targeting suppression of p53 protein may be beneficial to del(5q) MDS patients, who are resistant to lenalidomide [97].

BCOR and *BCORL1* are 2 transcriptional co-repressors located on chromosome X. BCOR protein has a BCL6 binding domain and acts as corepressor of BCL6. BCORL1 is a transcriptional corepressor binding to class II histone deacetylases to interact with the CTBP1 corepressor inducing E-cadherin repression. They were first discovered in AML by WES. Mutational analysis showed that *BCOR/BCORL1* mutations are found in 4.2% and 0.8% in MDS with higher frequency (6–9.1%) in AML or t-MDS patients [98]. BCOR mutations are associated with shorter survival (HR, 3.3; 95% CI, 1.4–8.1; *P*=0.008) [99].

5. RAS family pathway

Activating oncogenic mutations are rare in MDS. *N/K-RAS* were reported at 2–6% and associated with disease progression and shorter OS. RAP1, a member of the Ras family of small G proteins, were found mutated at low frequencies in MDS. One patient with high-risk MDS [RAEB with del(7q)] harbored 2 *RAP1B* mutations (G12R, K42E) [100]. GNAS (R201) was reported at 0.7% (3/439) in MDS [7]. Genetic abnormalities (mutations and amplification) in Ras-like-without-CAAX-1 (*RIT1*; 1q22) gene have been detected in high-risk MDS (8.4%) and in sAML (3.9%) [82].

6. Cohesin family

Genome-wide mapping clarified the cohesin complex structure and identified its role in chromosome condensation in Saccharomyces cerevisiae [101]. The structure of this complex resembles a ring constituted by 4 subunits (Scc1, Scc3, Smc1, Smc3) with the Smc1 and Smc3 being elements of the structural maintenance of chromosome family. Cohesins control that sister chromatids are connected during metaphase and segregate into right directions during cell division. Cohesins are important also in DNA-replication, DNA double-strand breaks repair, and chromosome condensation. Mutations in the cohesin complex (*STAG2, RAD21, SMC1A, SMC3*) have been found in 8.0% (18/224) of MDS, 12.1% (19/157) of AML, 10.2% (9/88) of CMML, and 6.3% (4/64) of chronic myeloid leukemia (CML). The role of cohesin in mitosis has been established, but its role in leukemogenesis is unclear. In 386 MDS cases, cohesin mutations were mainly enriched in trisomy 8 patients rather than in normal or complex karyotype. Mutations co-occur with *RUNX1*, Ras-family, *BCOR*, and *ASXL1*. *STAG2/RAD21* mutant responded better to HMT compared to WT patients (79% vs. 47%; *P*=0.04). Cohesin mutant patients had shorter OS compared to WT patients. (27.2 vs. 39.9 months; *P*=0.023) [102].

7. Less frequent molecular mutations

Genetic alterations in *SETBP1*, a gene associated with Schinzel-Giedion syndrome, were first found in 24% (17/70) of atypical CML patients [103] and then discovered in MDS. Somatic hererozygous mutations (Asp868, Ser869, Gly870, Ile871, Asp880) were detected in 17% of sAML and 15% of CMML [104]. *SETBP1* mutations are rare (2.2%) in MDS [105]. A study of 944 AML patients found 10 mutant patients (6 MDS; 4 sAML from MDS) [106]. *SETBP1* mutations are frequent in patients with myeloid neoplasms carrying i(17)(q10) and are associated with *SRSF2* mutations. *SETBP1* mutations tended to be associated with worse prognosis. Mutants were older and had higher monocyte counts compared to WT patients. Morphologycally, the cohort had prominent dysgranulopoiesis with hypo-/non-segmented nuclei [107]. A subsequent study conducted in 1,130 cases also found an association of *SETBP1* with i(17)(q10) and -7 abnormalities correlating *SETBP1* mutations with 2 other negative prognostic mutations (*ASXL1*, *CBL*) [108]. Mutations in *U2AF65, SF1, SRSF1, SF3A1, PRF40B,* and *LUC7L2* are infrequent [75].

NON-MOLECULAR ASPECTS OF MDS

Although molecular mutations are frequent in MDS, the consequences of these mutations have not been clarified. Other non-genetic mechanisms including BM-microenvironment factors, apoptosis, cytokines, immunoregulation, T-cell repertoire and telomere length (TL) have been largely studied. In supporting to the involvement of apoptotic pathways, death receptors (Fas, TRAIL), mitochondrial pathways and caspase activation have been found modulated in MDS cells. Indeed, MDS precursors overexpress Fas and TRAIL receptors, which seem to induce death signaling. Tumor necrosis factor alpha (TNF-α) also seems to be released by cytotoxic T-cells inducing apoptosis. Cytochrome c release is observed in low-risk MDS patients while caspase-9 activity is increased. Recently, it was reported that *SF3B1* mutant RARS/-T patients overexpress a specific variant of Mitoferrin-1, a mitochondrial iron importer [66]. The pro-apoptotic BCL2 protein has been found up-regulated in high-risk MDS and may explain the increased cellular proliferation [109]. Drugs (EPO, G-CSF) that target the apoptosis pathway improve the anemia in low-risk MDS.

Abnormalities in suppressive cytokines [TNF-α, tumor growth factor-β (TGF-β), interferon-γ (IFN-γ), interleukins-3, 6, and 8, thrombopoietin (TPO)] have been described in MDS. Overactivation of TGF-β depends on a family of proteins called Sma- and Mad-related (SMAD). SMAD2, a downstream regulator of TGF-β receptor I kinase activation, is constitutively activated in MDS $CD34^+$ cells, while SMAD7, a negative regulator, seems to be decreased in MDS cells [110]. Multiplex-analysis of cytokines/chemokines showed similarity between MDS and AML cells with a higher expression of the VEGF in MDS. High levels of TNF-α have been correlated with worse OS in MDS [111]. Higher levels of TPO and G-CSF, and lower levels of CD40L, CCL5, CCL11, VEGF, CXCL5, EGF, and CXCL11 were found by comparing plasma cytokines between aplastic anemia and MDS patients. Differences between low- and high-risk MDS were described with decreased CXCL5, CCL5, CD40L, EGF, and VEGF, and increased CCL4 in high-risk MDS [112]. HSCs in high-risk MDS patients are believed to proliferate bypassing the immune system. Differences in immune-complexes were found between low- and high-risk MDS and attributable to clonal progression. Cytotoxicity of BM cells in low-risk MDS has been associated with high numbers of NK cells, Th17, and macrophages releasing IFN-γ. $CD4^+FOXP3^+$-regulatory T cells have been correlated with immune response suppression and found elevated in high-risk MDS patients. The BM failure of MDS patients has been related to autoimmunity. In trisomy 8 MDS, spectratyping of T-cell receptor β-chain variable (Vβ) families revealed that $CD8^+$ T-lymphocytes are oligoclonal and selectively cytotoxic against trisomy 8 clones. Trisomy 8 clones seem to be resistant to the T-cell immune attack by up-regulating survivin. Microarray showed increased expression of WT1 in trisomy 8 $CD34^+$ cells [113].

Cellular senescence in MDS has been related to telomere shortening. BM MDS cells appear to have erosive telomeric repeats without changes in telomerase activity. Southern blot showed a variability in TL in MDS with a lower TL correlating with leukemic progression and complex cytogenetics [114]. Using multiplex-quantitative RT-PCR in 307 MDS patients, TL in BM cells was lower in MDS compared to healthy subjects with no correlation with age or gender. TL seems to be negatively correlated with IPSS, transfusion dependence, BM blasts and complex karyotype [115, 116]. Telomerase mutations/polymorphisms are sporadic in MDS. One patient with MDS and del(5q) harbored a *TERC* mutation [nucleotide 322 (G/A)] within the conserved region (CR) 4-CR5 domain, while 2 MDS patients carried polymorphisms [nucleotide 58 (G/A)] [117]. Mutations were found in the *TERC* and *TERT* genes in familial MDS/AML. The mutations affected the telomerase activity *in vitro*, and mutant patients had short telomeres [118]. Recently, a germline mutation (A377G) in the H box of the *TERC* gene was found in a young MDS patient with short telomeres [119].

Aberrant methylation is another important mechanism in MDS. Hypermethylation of promoter-CpG island of TSGs is a silencing mechanism and contributes to clonal evolution in MDS. Level of methylation also correlated with survival outcomes. One study showed that MDS patients with higher levels of aberrant CpG methylation in relatively common genes had a shorter median OS and PFS (OS: 12.3 vs. 17.5 months; P=0.04; PFS: 6.4 vs.14.9 months; P=0.009) compared to MDS patients with lower level of methylation [120].

CONCLUSION

MDS is a heterogeneous clonal disease with multifactorial causes. Molecular mutations in several pathways have been identified. Almost 78% of MDS patients carry at least one mutation in one gene. The presence of mutations has been associated with disease phenotypes and response to therapies. The use of NGS technologies has almost being implemented in clinical practice, although large clinical studies are needed to validate the possibility to use mutations as predictors of diagnosis, prognosis, and treatment response. Functional studies aim to decipher how genetic alterations may lead to the clinical phenotypes and whether targeting specific pathways or genes will be beneficial for MDS patients.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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