Review

Genetic basis of myelodysplastic syndromes

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Abstract: During the past decade, substantial progress has been made in the field of the genetics of myelodysplastic syndromes (MDS). These comprise a group of chronic myeloid neoplasms with abnormal cell morphology and progression to acute myeloid leukemia (AML), where revolutionary sequencing technologies have played a major role. Through extensive sequencing of a large number of MDS genomes, a comprehensive registry of driver mutations involved in the pathogenesis of MDS has been revealed, along with their impacts on clinical phenotype and prognosis. The most frequently affected molecules are involved in DNA methylations, chromatin modification, RNA splicing, transcription, signal transduction, cohesin regulation, and DNA repair. These mutations show strong positive and negative correlations with each other, suggesting the presence of functional interactions between mutations, which dictate disease progression. Because these mutations are associated with disease phenotype, drug response, and clinical outcomes, it is essential to be familiar with MDS genetics not only for better understanding of MDS pathogenesis but also for management of patients.

Keywords: myelodysplastic syndromes (MDS), splicing factors, cohesin, secondary acute myeloid leukemia (sAML), next-generation sequencing

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of chronic myeloid neoplasms, which are characterized by refractory cytopenia with abnormal bone marrow (BM) morphology and frequent progression to acute myeloid leukemia (AML) (secondary AML [sAML]).^{1,2)} Although AML progression, which is found in one-third of these patients, is a strong predictor of poor outcomes, many of the remaining patients also die from BM failure, which leads to fatal bleeding and infection.³⁾ Recently, several new drugs, such as lenalidomide⁴⁾ and hypomethylating agents,⁵⁾ have been introduced into clinics, contributing improved outcomes for a subset of MDS patients. However, no curative therapy is available for MDS patients, except for allogeneic hematopoietic stem cell transplantation,

which unfortunately is applicable only to younger patients, even though the vast majority of patients are elderly and not eligible to transplantation. Thus, there is an urgent need to understand the molecular pathogenesis of MDS to develop effective but less toxic therapies.

Little was known about relevant gene mutations that are implicated in MDS pathogenesis until early 2000s. This was in contrast to AML, whose molecular pathogenesis was intensively studied during the 1990s through the analysis of highly recurrent chromosomal translocations. In fact, only a handful of genes, including *NRAS*, *TP53*, *ATRX*, and *RUNX1*, were known to be mutated in MDS.^{6)–10)} The first breakthrough was brought about by the development of single nucleotide polymorphism (SNP) array-based genomic copy number analysis (or SNP array karyotyping), using millions of SNP probes, which enabled the comprehensive detection of genomic copy number abnormalities (CNAs) at an unprecedented resolution (~several kilobase pairs) across the entire cancer genome.^{11),12)} Thus, using SNP-array karyotyping, we and other groups identified a number of recurrent deletions, amplifications,

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and other allelic imbalances, such as copy neutral loss-of-heterozygosity (LOH) or uniparental disomies (UPDs),¹³⁾ from which novel mutational/functional target genes were identified, including *TET2*,^{14),15)} *CBL*,¹⁶⁾ *EZH2*,^{17),18)} *CUX1*,¹⁹⁾ and *ASXL1*,²⁰⁾ which are targeted by common copy number lesions in 4q (UPD/deletion), 11q (UPD), 7q (UPD/deletion), and 20q (deletion), respectively. Other gene targets of common CNAs include *NRAS* and *cMPL* (1pUPD), *FLT3* (13qUPD), *TP53* (17qUPD/deletion), and *RUNX1* (21qUPD/deletion).¹⁶⁾

It was not until the revolution in sequencing technologies were introduced into the genetic analysis of cancer that the entire picture of genetic alterations in MDS have been revealed and confirmed in large cohorts of MDS patients; followed by the first report of sequencing of an entire AML genome in 2008.²¹⁾ Next-generation sequencing has been used for analyses of major human cancers, including MDS, to unveil the complete landscape of mutations and other genetic lesions.^{22),23)} The power of this technology is highlighted by the discovery of mutations in *DNMT3A* and *IDH1* on the basis of whole genome sequencing of an index AML case, which turned out to be among major mutations not only in AML but also in MDS.^{24),25)}

Identification of splicing factor mutations in MDS using whole exome sequencing

To investigate novel mutational targets in MDS in an unbiased manner, we performed whole exome sequencing analysis of paired tumor/normal DNA from 29 cases with MDS and related myeloid neoplasms, including chronic myelomonocytic leukemia and sAML. On average, we identified 265 mutations (9 somatic mutations/sample).²⁶⁾ Most of the mutated genes were affected in a single sample, except for 13 genes shown in Table 1 (in bold), which were recurrently mutated in multiple samples, suggesting their role in MDS pathogenesis. Although 8 of these genes were well-known mutational targets in MDS, the remaining 4 were not reported previously, of which three genes (*U2AF1*(*U2AF35*), *SRSF2*, and *ZRSR2*) encode RNA splicing factors (SFs). Notably, also including an additional three genes (*SF3A1*, *SF3B1*, and *PRPF40B*), 16 out of 29 MDS cases analyzed had a mutated SF, suggesting that deregulated RNA splicing plays a central role in MDS pathogenesis.

RNA splicing is a basic cellular apparatus ubiquitously found in all eukaryotes, which generates a wide variety of different splicing forms, enabling

high-degrees of protein species expressed in eukaryotic organisms.²⁷⁾ The process begins with recognition of exon/intron boundaries, which is accomplished by U1 or U2 snRNP complex, followed by two trans-ester reactions and excision of the intronic sequence as a lariat structure. Of interest, except for PRPF40B, whose function is not well-established, all SFs mutated in 29 cases are included in the U2 RNP complex and involved in one of the initial steps of mRNA splicing, *i.e.*, 5' splice site recognition (Fig. 1a). To confirm these initial results from whole exome sequencing in the 29 cases, we performed extended mutation analysis in a large cohort of 582 cases with a wide variety of myeloid neoplasms, including not only different subtypes of MDS and sAML, but also *de novo* AML as well as classical myeloproliferative neoplasms, focusing on SFs involved in 5' splice site recognition. Through this large-scale analysis, we confirmed that SF mutations were found in all subtypes of MDS, such as refractory anemia (RA), RA with excess blasts (RAEB), refractory cytopenia with multilineage dysplasia (RCMD) as well as RA with ringed sideroblasts (RARS) and refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS), and also chronic myelomonocytic leukemia (CMML) at very high frequencies ranging from 45% to 85%, depending on subtype (Fig. 1b). Conspicuously, in two unique subtypes, RARS and RCMD-RS, which were characterized by elevated numbers of ring-sideroblasts (also known as sideroblastic anemia), >70% of the patients had *SF3B1* mutations, making these mutations almost pathognomonic to these MDS subtypes, which was also confirmed in other studies.^{28),29)} Moreover, SF mutations were also found in other myeloid neoplasms, including *de novo* and secondary AML as well as classical myeloproliferative neoplasm (MPN) cases, although mutation frequencies were largely <10%, except in sAML progressed from MDS, where an intermediate mutation frequency (~25%) was observed. These findings strongly suggest that SF mutations are cardinal features of MDS and to a lesser extent, other myeloid neoplasms.³⁰⁾

SF mutations were found in at least 8 SFs, of which by far the most frequently affected were *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*.²⁶⁾ Among these, mutations in *SF3B1*, *SRSF2*, and *U2AF1* showed prominent mutational hotspots (Fig. 1c); in *SRSF2* most mutations affected the P95 residue between two functional domains, whereas mutations in *U2AF1* exclusively affected two highly conserved

Table 1. Genes mutated in multiple samples

Gene	Type	Chr	Mutation	A.A	Sample ID
<i>ASXL1</i>	Nonsense	Chr20	C > A	S852X	MDS-19
<i>ASXL1</i>	Nonsense	Chr20	C > T	R693X	CMML-01
<i>BCOR</i>	Frameshift	ChrX	*/+C	V1351fs	MDS-19
<i>BCOR</i>	Nonsense	ChrX	C > T	Q1187X	CMML-02
<i>DNMT3A</i>	Missense	Chr2	G > T	G543C	MDS-19
<i>DNMT3A</i>	Missense	Chr2	G > A	R882H	CMML-02
<i>EZH2</i>	Missense	Chr7	A > C	D664A	tAML-01
<i>EZH2</i>	Frameshift	Chr7	*/-AA	K718fs	CMML-01
<i>KRAS</i>	Missense	Chr12	G > A	G12D	tAML-06
<i>KRAS</i>	Missense	Chr12	A > G	K5E	CMML-01
<i>NRAS</i>	Missense	Chr1	G > A	G13D	MDS-19
<i>NRAS</i>	Missense	Chr1	G > A	G13D	MDS-20
<i>NRAS</i>	Missense	Chr1	G > A	G12S	MDS-20
<i>NRAS</i>	Missense	Chr1	G > A	G12S	tAML-05
<i>RUNX1</i>	Frameshift	Chr21	*/+G	L294fs	MDS-19
<i>RUNX1</i>	Missense	Chr21	G > A	D171N	tAML-02
<i>SRSF2</i>	Missense	Chr17	C > T	P95L	MDS-09
<i>SRSF2</i>	Missense	Chr17	C > A	P95H	MDS-18
<i>SRSF2</i>	Missense	Chr17	C > A	P95H	CMML-04
<i>SRSF2</i>	Deletion	Chr17	—	P95HR103>R	tAML-07
<i>STAG2</i>	Nonsense	ChrX	C > T	R1012X	MDS-12
<i>STAG2</i>	Splice site	ChrX	G > A	Q399_spl	MDS-19
<i>TET2</i>	Frameshift	Chr4	*/-C	I340fs	MDS-06
<i>TET2</i>	Frameshift	Chr4	*/+C	Y1255Fs	MDS-16
<i>TET2</i>	Nonsense	Chr4	C > T	Q323X	MDS-16
<i>TET2</i>	Nonsense	Chr4	C > G	S825X	MDS-19
<i>TET2</i>	Frameshift	Chr4	*/+A	L1046fs	MDS-19
<i>TET2</i>	Nonsense	Chr4	C > T	R550X	tAML-01
<i>TET2</i>	Frameshift	Chr4	*/-C	H994fs	tAML-01
<i>TET2</i>	Nonsense	Chr4	C > T	Q1680X	tAML-07
<i>TET2</i>	Frameshift	Chr4	*/+A	S402Fs	tAML-07
<i>TET2</i>	Frameshift	Chr4	*/-AAAT	Q749fs	CMML-01
<i>TP53</i>	Missense	Chr17	C > T	R116W	MDS-09
<i>TP53</i>	Frameshift	Chr17	*/-T	M1fs	tAML-03
<i>U2AF1</i>	Missense	Chr21	A > G	Q157R	MDS-03
<i>U2AF1</i>	Missense	Chr21	C > T	A26V	MDS-12
<i>U2AF1</i>	Missense	Chr21	A > C	Q157P	MDS-15
<i>U2AF1</i>	Missense	Chr21	A > G	Q157R	CMML-01
<i>U2AF1</i>	Missense	Chr21	C > T	S34F	CMML-02
<i>ZRSR2</i>	Splice site	ChrX	G > A	K257splice	MDS-06
<i>ZRSR2</i>	Frameshift	ChrX	*/-G	G323fs	MDS-08
<i>ZRSR2</i>	Nonsense	ChrX	G > T	E362X	MDS-16
<i>ZRSR2</i>	Missense	ChrX	A > T	N261Y	tAML-05

amino-acids within Zinc-finger domains flanking the RNA binding domain (S34 and Q157).^{26),31),32)} In addition, in *SF3B1* nearly half of the mutations affected K700 and surrounding amino-acids.²⁶⁾ The

presence of these prominent mutational hot spots strongly suggested that these cause neomorphic or gain-of-function mutations. By contrast, mutations in *ZRSR2* were distributed along the entire protein,

Fig. 1. Splicing factor mutations in MDS and related neoplasms. **a**. SFs involved in the recognition of 3' (U1 complex) and 5' (U2 complex) splice sites. SFs mutated in MDS and other myeloid malignancies are shown as arrows. **b**. Frequency of SF mutations in different subtypes of MDS and other myeloid malignancies. **c**. Distribution of SF mutations in major SFs. Red arrowheads indicate confirmed somatic mutations. Zn: Zinc finger domain, RS: arginine/serine rich domain. **d**. Mutually exclusive pattern of mutations in myeloid neoplasms, in which samples are shown in horizontal columns. Cited from Yoshida *et al.*, Nature, 2011.²⁶⁾

most of which resulted in a premature truncation, and thus were thought to cause loss-of-function. Additional important features of SF mutations in MDS and other myeloid neoplasms are that these mutations occurred in a mutually exclusive manner and that except for *ZRSR2* mutations, all SF mutations were heterozygous with no homozygous mutations (Fig. 1d).²⁶⁾ These features suggest that multiple or homozygous SF mutations were lethal and not compatible with cell viability and positive selection of cells having SF mutations in such a manner.

Functional consequence of SF mutations

Several studies, including ours, addressed the effects of SF mutations on RNA splicing^{33)–41)} and biological phenotypes.^{36),38),42)–45)} To investigate the effects of SF mutations on RNA splicing, we performed RNA sequencing of CD34+ cells from a total of 100 MDS patients, including 32 cases with *SF3B1* mutations and 23 with *SRSF2* mutations. RNA from whole BM cells were also sequenced for 165 patients, which consisted of 54 *SF3B1*-mutated

and 32 *SRSF2*-mutated, and 14 *U2AF1*-mutated cases.^{34),40)} As expected, SF-mutated samples showed extensive changes in splicing compared with samples without any SF mutations ($n = 41$ for CD34+ cells and $n = 66$ for whole BM cells) (Fig. 2a). However, the patterns of splicing changes differed substantially depending on the mutated SF. For example, *SF3B1*-mutated samples showed significantly increased alternative 3' splice sites, which were 5' upstream of a canonical branch point, suggesting that they were caused by the recognition of an alternative 5' branch point by the mutated *SF3B1*.^{34),40)} *SF3B1*-mutated samples also exhibited a dramatically reduced number of retained introns (Fig. 2a).^{34),40)} Of note many of these alternative splicing forms in *SF3B1*-mutated samples cause nonsense-mediated decay, leading to significantly reduced gene expression (Fig. 2b). A notable example is 3' alternative splicing in *ABCB7* encoding an iron transporter in mitochondria, haploinsufficiency of which is responsible for congenital sideroblastic anemia (Fig. 2c).^{34),40),46)} By contrast, mutations in *SRSF2*, which binds to exonic splicing enhancer (ESEs) via

Fig. 2. (Color online) Abnormal splicing in SF-mutated cells. **a**. Types and numbers of splicing alterations significantly associated with each SF mutation. Bars in the upper half indicate alternative splicing events that were more frequently found in SF-mutated cases. Bars in the lower half are those that were more frequently found in the controls. Types of alternative splicing events are shown on the right, where cassette exon means inclusion or skipping of an exon. **b**, **e**. Volcano plots comparing expression levels of authentic transcripts between the (**b**) *SF3B1*- and (**e**) *SRSF2*-mutated CD34⁺ cells and those without known SF mutations. The number of samples in each group is shown in Fig. 1c. X-axis indicates fold changes in gene expression on a log₂ scale. Y-axis indicates *q*-values on a negative log₁₀ scale. Target genes of mutant *SF3B1*-associated alternative 3' splice sites are depicted in red (**b**). Those of mutant *SRSF2*-associated cassette exons are depicted in blue (**e**). **c**. Increased 3' alternative splice site (3'ss) at exon 8/9 junction in *ABCB7* in *SF3B1*-mutated cells compared with *SF3B1*-wild type cells, which becomes prominent by cyclohexamide (CHX) treatment. **d**. Forest plots show relative frequencies of CCNG and GGNG motifs within the differentially spliced exons in the *SRSF2*-mutated samples. Two panels show the exons that are more frequently included (*n* = 380) (top) and skipped (*n* = 645) (bottom) in *SRSF2*-mutated samples, respectively. Comparisons were made between the differentially spliced exons and constitutive ones (*n* = 72,002). Red and blue dots indicate relative frequencies of CCNG and GGNG, respectively. Bars indicate 95% confidence intervals. Relative frequencies are shown for exons divided into four segments with equal lengths. **f**. Two cassette exons in *EZH2*-associated with *SRSF2* mutation. The left is alternative exon usage and the right is exon skipping. Gray boxes indicate constitutive exons. Red and blue boxes denote exons with increased and decreased usage in *SRSF2*-mutated samples, respectively. Cited from Shiozawa *et al.*, Nature Communications, 2018.⁴⁰

consensus sequences (SSNG) and is implicated in the regulation of splicing efficiency, were associated with enhanced or suppressed cassette (or alternative) exons, where alternative exons containing the CCNG consensus have higher affinity to mutant *SRSF2* tended to be more frequently included, whereas those carrying the consensus with less affinity were more often excluded (Fig. 2d).^{36,40} Splicing changes in *U2AF1*-mutated samples were dominated by alter-

ations in cassette exons as seen in *SRSF2*-mutated samples. In contrast to a 3' alternative splice site, alternative cassette exons seen in *SRSF2* or *U2AF1* mutants were less likely to influence gene expression. Nevertheless, some genes with mutant *SRSF2*- or *U2AF1*-associated alternative splicing resulted in reduced gene expression (Fig. 2e). An example is *EZH2*, a catalytic component of PRC2 frequently mutated in MDS, whose alternative exons in introns

10 and 11 are more frequently included or excluded caused a premature termination in *SRSF2*-mutated cells, of which the cassette exon in intron 10 is also included in the U2AF1 S34F mutants (Fig. 2f). Alternative splicing affecting *EZH2* provides an example in which different SF mutants affected splicing of a common target, which was rarely seen despite large numbers of gene targets of SF mutant-associated alternative splicing. However, in general there were a few genes commonly mutated in MDS whose alternative splicing was caused by different SF mutants.

We and other groups also investigated the biological consequences of mutant SFs using an engineered mouse model with conditional mutant SF alleles. However, even though targets of abnormal splicing of SF mutations overlapped with known mutational targets, the molecular pathogenesis of mutated SFs has not fully been elucidated. Indeed all these mouse models showed abnormal splicing of expected patterns, as seen in human samples, and developed dysplastic cell morphology as well as reduced production of mature blood cells. However, BM cells from these mice did not show any evidence of clonal advantage in competitive repopulation analysis; when equivalent numbers of BM cells from mutant and wild-type mice were transplanted into lethally irradiated recipients. When chimerism between wild-type and mutant SFs was evaluated, mutant cells unexpectedly showed a reduced rather than elevated chimerism, suggesting that SF-mutant BM cells have compromised repopulation capacity. One of the explanations might be an altered BM microenvironment favoring SF-mutant cells. Future studies are clearly warranted to clarify the exact mechanism of positive selection of SF-mutant cells in MDS patients.

Cohesin mutations

An additional mutational targets mutated in MDS more frequently than expected only by chance (*i.e.* driver genes) were identified through exome sequencing of an extended cohort. Cohesin is a multimeric protein complex comprising RAD21, SMC1, SMC3, and a STAG protein (STAG1-3) (Fig. 3a).⁴⁷⁾ Taking a ring-like structure, these molecules are recruited on chromatin in concert with cohesin-associated molecules, such as NIPBL and ESCO proteins, to prevent sister chromatids from premature separation during cell division. However, these also participate in post-replicative DNA repair and long-range regulation of gene expression through

the formation of large-scale chromatin structures (Fig. 3b).^{48),49)} In MDS and other myeloid malignancies, approximately 10–15% of cases harbor mutations in cohesin and related molecules.^{50),51)} Among these cohesin components, STAG2 was most frequently mutated, in which most mutations were truncating, leading to loss-of-function, and so were RAD21 mutations (Fig. 3c). As is the case with SF mutations, cohesin mutations are largely mutually exclusive (Fig. 3d), suggesting synthetic lethality of mutations in multiple cohesin components. Cohesin mutations were also reported in several solid tumors, including glioblastoma, melanoma, Ewing sarcoma, and colon cancers, where aneuploidy caused by loss of cohesin functions has been implicated in tumorigenesis.⁵²⁾ However, in myeloid malignancies, many of cohesin-mutated tumors had normal or near normal karyotypes (Fig. 3d), arguing against the role of aneuploid but supporting altered gene regulation as the mechanism of cohesin-mutated myeloid neoplasms. Recent studies showed that cohesin-deficient cells had altered chromatin structures that allowed for accessibility to a number of transcription factors, including RUNX1, GATA2, and ERG, suggesting functional interactions of cohesin mutations and these transcription factors.⁵³⁾

Landscape of gene mutations in MDS

Other newly identified driver genes in MDS include *BCOR/BCORL1*,^{26),54)} *BRCC3*,^{55),56)} *PHF6*,^{26),57)} *ETV6*, *SETBP1*,^{58),59)} *PRPF8*,⁶⁰⁾ and *RIT1*.⁶¹⁾ Thus, to elucidate the landscape of mutations in MDS, we conducted a comprehensive analysis of mutations in these major driver or candidate driver genes in a total of 944 MDS patients using targeted-capture sequencing. In total 47 genes were mutated at frequencies expected only by chance, where one or more of the 47 driver genes were mutated in as many as 864 (91.5%) of the 944 patients (Fig. 4).⁵⁵⁾ These driver genes were grouped into several discrete functional pathways, including RNA splicing, DNA methylation (*TET2*, *DNMT3A*, *IDH1* and *IDH2*), chromatin modification (*ASXL1*, *EZH2*, and *KDM6A*), transcription (*RUNX1*, *ETV6*, *BCOR/BCORL1*, *GATA2*, *CEBPA*, and *NPM1*), receptor/tyrosine kinase, cohesin, RAS signaling (*KRAS*, *NRAS*, *PTPN11*, and *CBL*), and DNA repair (*ATM*, *BRCC3*, and *FANCL*). With a median of 3 per sample, the number of driver mutations increased with WHO risk. Among these, only 6 driver genes were mutated at 10% $\geq$ frequencies, followed by many low-frequency mutated genes. Most of the

Fig. 3. (Color online) Mutations in cohesin complex. **a**. Structure of the cohesin complex comprising SMC1, SMC3, RAD21, and one of STAG1-3 proteins. Taking a ring-like structure, cohesin is loaded onto two chromatin strands in concert with cohesin-associated molecules. **b**. Cohesin is thought to be involved in gene regulation through interaction with transcription factors and other transcriptional apparatus. **c**. Distribution of mutations in major cohesin components. **d**. Mutually exclusive distribution of mutations of different cohesin components. Cited from Kon *et al.*, Nature Genetics, 2013.⁵¹⁾

driver genes commonly mutated in MDS were also mutated in AML, but their frequency is substantially different between these malignancies (Fig. 5),⁶²⁾ suggesting that distinct pathogenesis between MDS and AML despite a substantial overlap in mutated drivers. In particular, a number of driver genes, including *NRAS*, *FLT3*, *KRAS*, *PTPN11*, *IDH1*, *IDH2*, *WT1*, *RAD21*, *KIT*, and *CEBPA*, were less frequently mutated in MDS compared with in primary AML (Fig. 5).⁶³⁾ This was also in line with the differences in the cytogenetic or copy number

alteration profiles between AML and MDS. AML is characterized by frequent disease-specific reciprocal translocations, such as *t*(8;21)(q22;q22), *inv*(16)(p13q22), *t*(3;21)(q26;q22), *t*(15;17)(q22;q21) and other *MLL*(11q23)-affecting translocations, leading to *RUNX1/RUNX1T1*, *RUNX1/EVI1*, *PML/RARA*, and a variety of *MLL* fusions, respectively. By contrast, most of the chromosomal lesions in MDS are unbalanced changes, which are caused by deletion and amplification of one or more chromosomal segments, including *del*(5q)/-5, *del*(7q)/-7, 12pLOH,

Fig. 4. (Color online) Landscape of gene mutations in 944 MDS cases. Mutations in 47 genes significantly mutated in 944 MDS cases are depicted. Driver genes are clustered according to their functions. Samples are shown in the horizontal axis with WHO classification in the indicated colors. Cited from Haerlach *et al.*, Leukemia, 2014.⁵⁵⁾

del(20q), and 17pLOH (Fig. 5), most of which are seen in combinations as complex karyotypes in association with biallelic *TP53* involvement. $t(3;21)(q26;q22)$, $inv(3)(q21q26)$, $t(3;3)(q21;q26)$, $t(1;3)(q36;q21)$, as well as $t(6;9)(q22;q34)$ are typically seen in borderline cases between AML and MDS, predicting poor prognosis.⁶³⁾ Other abnormalities, such as +8, +21, are also seen in both AML and MDS at similar frequencies.

Co-occurring and mutually exclusive mutations

Among these driver mutations and copy number alterations, there exist strong positive and negative correlations. By exhaustively interrogating pairwise combination of mutations among the 944 patients, we detected a total of 82 significant ($q < 0.01$) combinations of mutations (Fig. 6), suggesting strong functional interactions between these mutations. Most of these significant combinations of mutations showed excellent concordance with those detected by analysis of independent MDS cohorts

(Table 2), confirming the validity of these mutational combinations.⁶⁴⁾ For example, SF mutations are almost mutually exclusive with each other and so are mutations in components of the cohesin complex, likely due to synthetic lethality of multiple mutations in these pathways and complexes. By contrast, *STAG2* mutations significantly cooccurred with mutations in *RUNX1*, *SRSF2*, *ASXL1*, *EZH2*, *BCOR*, and *IDH2*.^{55),64)} Mutations and copy number alterations are largely mutually exclusive with some exceptions, of which significant correlations between copy number alterations and *TP53* mutations. *TP53* mutations, particularly when biallelic, are tightly associated with complex karyotypes with aneuploidy, which uniformly shows very poor prognosis resistant even to allogeneic stem cell transplantation, whereas *TP53* mutations tend to be mutually exclusive with other mutations (Fig. 6).⁶³⁾

Moreover, not only combinations of mutations but also their order of acquisition is not totally random, but there is a strong trend that some

Fig. 5. Different mutation frequency between MDS, sAML, and primary AML. Frequency of major driver genes mutated in common in different myeloid malignancies, including MDS, sAML, and *de novo* primary AML. Risks of MDS are indicated by color. Mutations commonly seen in primary AML but only rarely in MDS are shown in dark blue in the AML panel. Cited from Ogawa, Blood, 2019.⁶³⁾

mutations tend to precede others, frequently associated with progression from low-risk to high-risk diseases and to sAML. To examine this, we interrogated significant enrichment of mutations between low- and high-risk MDS and also between high-risk MDS and sAML in a cohort of 2,250 MDS cases.⁶⁵⁾ As shown in Fig. 7a, there was a significant enrichment of *SF3B1* mutations in low-risk *vs.* high-risk MDS cases, whereas mutations in *TP53*, *GATA2*, *RUNX1*, *KRAS*, *STAG2*, *ASXL1*, *ZRSR2*, and *TET2* (Type-2 mutations) were significantly enriched in high-risk compared with low-risk MDS. Between high-risk and sAML cases, there was a significant enrichment of mutations in *FLT3*, *PTPN11*, *WT1*, *IDH1*, *NPM1*, *IDH2*, and *NRAS*

(Type-1 mutations) (Fig. 7a). These results suggested that Type-2 and Type-1 mutations are associated with progression from low-risk to high-risk MDS and high-risk MDS to sAML, respectively. To see this, we investigated the impact of Type-1 and Type-2 mutations on progression to sAML. We found that patients carrying one or more of Type-1 mutations at the time of diagnosis tended to progress to sAML earlier than those cases with Type-2 mutations alone (Fig. 7b). Patients with *SF3B1* mutations but not Type-1 or Type-2 mutations rarely progressed to sAML and those without of Type-1 or Type-2 mutations displayed an intermediate risk of AML progression. Notably, all Type-1 mutations were also strongly enriched in primary

Fig. 6. Genetic interactions between major driver mutation. Correlation-coefficients and corresponding q value are shown by color gradient and size of circle for all combinations of mutations in the indicated major drivers in 944 MDS cases. *TP53* mutation and CNAs are shown in red and blue, respectively. Cited from Ogawa, Blood, 2019.⁶³⁾

AML, compared with MDS.⁶³⁾ Of further interest, upon progression to sAML, acquisition of one or more Type-1 mutations was confirmed in some patients with Type-2 mutations alone. These Type-1 and Type-2 mutations demonstrated significant co-occurrence between each other, suggesting that mutations are associated with disease progression. Recent studies more closely described the dynamics of clones during progression from MDS to AML using longi-

tudinal samples for deep sequencing and single-cell sequencing. The presence of multiple clones in MDS, particularly in immature stem cell fractions, from which a clone evolves to sAML, while swiping other clones.^{65)–67)}

Clinical impact of mutations

Finally, we evaluated the impact of mutations on survivals of 944 MDS patients. To examine the

Table 2. High concordance of coexisting mutations between independent studies

	Haferlach et al., Leukemia 2014 ⁵⁵⁾ (N = 944)	Papaemmanuil et al., Blood 2013 ⁶⁴⁾ (N = 738)
<i>TET2</i>	<i>ZRSR2</i> (q < 0.0001), <i>SRSF2</i> , <i>CBL</i> (q < 0.001)	<i>SRSF2</i> , <i>ZRSR2</i> (q < 0.01)
<i>SF3B1</i>	<i>DNMT3A</i> , <i>JAK2</i> (q < 0.01)	<i>DNMT3A</i> (q < 0.01)
<i>SRSF2</i>	<i>STAG2</i> , <i>ASXL1</i> , <i>RUNX1</i> , <i>IDH2</i> (q < 0.0001), <i>TET2</i> (q < 0.001), <i>CBL</i> (q < 0.01)	<i>STAG2</i> , <i>IDH2</i> , <i>CUX1</i> , <i>ASXL1</i> , <i>TET2</i> , <i>RUNX1</i> (q < 0.01)
<i>U2AF1</i>	<i>ASXL1</i> (q < 0.0001), <i>PHF6</i> , <i>ETV6</i> (q < 0.01)	<i>ASXL1</i> (q < 0.05)
<i>ZRSR2</i>	<i>TET2</i> (q < 0.0001), <i>PHF6</i> (q < 0.01)	<i>TET2</i> (q < 0.01)
<i>STAG2</i>	<i>ASXL1</i> , <i>RUNX1</i> , <i>SRSF2</i> , <i>NRAS</i> , <i>BCOR</i> (q < 0.0001), <i>IDH2</i> (q < 0.001), <i>EZH2</i> (q < 0.01)	<i>SRSF2</i> , <i>RUNX1</i> (q < 0.01), <i>ASXL1</i> (q < 0.05), <i>IDH2</i> , <i>EZH2</i> (q < 0.1)
<i>DNMT3A</i>	<i>SF3B1</i> (q < 0.001)	<i>SF3B1</i> (q < 0.01), <i>BCOR</i> (q < 0.05)
<i>RUNX1</i>	<i>SRSF2</i> , <i>ASXL1</i> , <i>EZH2</i> , <i>STAG2</i> , <i>BCOR</i> (q < 0.0001)	<i>STAG2</i> , <i>ASXL1</i> , <i>SRSF2</i> (q < 0.01), <i>EZH2</i> (q < 0.05), <i>NRAS</i> (q < 0.1)
<i>ASXL1</i>	<i>SRSF2</i> , <i>U2AF1</i> , <i>STAG2</i> , <i>IDH2</i> , <i>EZH2</i> , <i>RUNX1</i> , <i>NRAS</i> (q < 0.0001), <i>CBL</i> (q < 0.01)	<i>SRSF2</i> , <i>RUNX1</i> (q < 0.01), <i>JAK2</i> , <i>U2AF1</i> , <i>STAG2</i> (q < 0.05), <i>IDH2</i> , <i>NRAS</i> , <i>CUX1</i> (q < 0.1)
<i>EZH2</i>	<i>RUNX1</i> , <i>ASXL1</i> , <i>TP53</i> , <i>LAMB4</i> , <i>LUC7L2</i> , <i>NPM1</i> , <i>ETV6</i> (q < 0.0001), <i>NRAS</i> (q < 0.001), <i>STAG2</i> (q < 0.01)	<i>RUNX1</i> (q < 0.05), <i>STAG2</i> (q < 0.1)

Significant correlations in both studies are shown in bold.

impact of gene mutations, we generated two Cox proportional hazard models, in which the model was constructed using mutation alone (Model-II) or both clinical factors and mutations (Model-I), including complete blood counts, BM blast%, and cytogenetics, which were compared with IPSS and IPSS-R conventionally used for prognostication of MDS patients, using LASSO for selection of variables. In univariate analysis, mutations in 25 genes were significantly associated with overall survival (OS), of which only SF3B1 mutations predicted a better OS. The model combining clinical and gene mutations significantly outperformed traditional IPSS and IPSS-R models, which was in agreement with other studies,⁵⁵⁾ but in accordance of an earlier studies evaluating the clinical effects of mutations in MDS.⁶⁸⁾

Conclusions

During the past two decades, our knowledge about the genetics of MDS has improved dramatically, with the revolution in genomics, particularly next-generation sequencing, providing a comprehensive registry of driver mutations in MDS, their frequency, and mutual correlations, as well as their impacts on disease phenotypes and prognosis. Now, allied efforts are in progress by the international

working group for the prognosis of MDS (IWG-PM), in which thousands of MDS patients are being investigated for major driver mutations to confirm and further extend these initial findings. These studies provide a solid basis for novel molecular classifications of MDS, which help accurate diagnosis and prognostication, as well as better choice of therapies to ultimately improve patients' clinical outcomes. Finally, despite a large number of newly revealed driver mutations, their functional roles in MDS pathogenesis remain to be elucidated for many drivers. For example, as seen above, SF mutations unequivocally play critical roles in MDS pathogenesis, whereas no functional evidence has been demonstrated for how SF-mutated hematopoietic cells achieve a clonal dominance; SF-mutated stem cells show a compromised competitive repopulation potential over normal stem cells in mouse models.^{36),42)–45)} The importance of understanding of the functional/molecular mechanisms of these driver mutations identified through genetic analyses cannot be underestimated.

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Fig. 7. (Color online) Major role of driver mutation in disease progression. **a.** Enrichment of driver mutations in sAML and high-risk MDS relative to high-risk and low-risk MDS, respectively. Enrichment is expressed as an odds ratio (OR) of mutation rates in sAML ($n = 360$) *vs.* high-risk MDS ($n = 683$) on the x axis and high-risk ($n = 683$) *vs.* low-risk ($n = 1,207$) on the y axis. Logistic regression analysis was applied to 25 driver genes measured in whole cohorts in 2,250 MDS and sAML patients, and the best model was selected by the least absolute shrinkage and selection operator. Mutations showing significant enrichment in either comparison are indicated by colors according to OR 95% CI limits being above (if $OR > 1$) or below (if $OR < 1$). According to their distinct enrichment patterns, mutations are classified into Type 1 or Type 2, as indicated. **b.** Progression-free survival of 429 patients with Type-1 mutations (group I) with Type-2 but no Type-1 or Type-2 mutations (group II), with *SF3B1* but no Type-1 or Type-2 mutations (group III), and other patients with no Type-1, Type-2, or *SF3B1* mutations (group IV). Cited from Makishima *et al.*, Nature Genetics, 2017.⁶⁵⁾

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Profile

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