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Novel Therapeutic Strategies in MDS: Do Molecular Genetics Help?

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Abstract

Purpose of Review—Many studies over the past decade have together identified genes that are recurrently mutated in the myelodysplastic syndromes (MDS). We will summarize how this information has informed our understanding of disease pathogenesis and behavior, with an emphasis on how this information may inform therapeutic strategies.

Recent findings—Genomic sequencing techniques have allowed for the identification of many recurrently mutated genes in MDS, with the most common mutations being found in epigenetic modifiers and components of the splicing machinery. While many mutations are associated with clinical outcomes and disease phenotypes, at the current time they add relatively little to already robust clinical prognostic algorithms. However, as molecular genetic data is accumulated in larger numbers of patients, it is likely that the clinical significance of co-occurring mutations and less common mutations will come to light. Finally, mutated genes may identify biologically distinct subgroups of MDS that may benefit from novel therapies, and a subset of these genes may themselves serve as therapeutic targets.

Summary—Advances in our knowledge of the molecular genetics of MDS have significantly improved our understanding of the disease biology and promise to improve tools for clinical decision-making and identify new therapies for patients.

Keywords

The myelodysplastic syndromes; myelodysplasia; molecular genetics; prognostic models; novel therapeutics

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Conflicts of Interest:

There are no relevant conflicts of interest to disclose.

Introduction

The myelodysplastic syndromes (MDS) exemplify the heterogeneous nature of myeloid malignancies, both in their clinical behavior and underlying disease biology. In recent years, advances in genomic sequencing technologies have allowed for the identification of a plethora of somatic mutations in MDS that have yielded immense insight into disease biology. Moreover, many newly identified disease alleles are associated with disease phenotypes and clinical outcomes. Nevertheless, the incorporation of molecular genetic information into diagnostic and prognostic algorithms has yet to enter routine clinical practice, and novel therapies based on this information remain lacking. Herein we review recent advances in our understanding of the molecular genetics of MDS, with a particular focus on how these advances may directly influence clinical practice and novel therapeutic strategies.

Epigenetic Modifiers

Aberrant DNA methylation has long been observed as a feature of MDS that may be predictive of prognosis and disease progression^{1–7}, and therapy with DNA-methyltransferase inhibitors such as 5-azacytidine and decitabine can effect robust clinical responses^{8–10}. Thus, it is not surprising that epigenetic modifiers have been found to be among the most commonly recurrently mutated genes in MDS.

In 2009, high-resolution single nucleotide polymorphism and comparative genomic hybridization arrays were utilized to identify in patients with myeloid malignancies a minimally deleted region on chromosome 4q24 encompassing *TET2*¹¹. Subsequent targeted sequencing studies identified *TET2* mutations in 19–26% of MDS cases^{12–15}. Simultaneously, *TET2* was discovered to have a function in the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC)¹⁶, leading to subsequent demethylation of DNA^{17–19}, providing a link between these mutations and aberrant methylation.

A frame-shift mutation in *DNMT3A* was identified in a re-analysis of the first sequenced acute myeloid leukemia (AML) genome²⁰, and subsequent analyses of MDS patients identified *DNMT3A* mutations at a frequency ranging from 3–13% of cases^{21–24}. The presence of *DNMT3A* in nearly all of the cells of the BM of MDS patients²² suggests its role as a founding mutation, further bolstered by recent studies implicating *DNMT3A* as a pre-leukemic mutation^{25–27}.

Sequencing of another AML genome identified a mutation in *IDH1*²⁸, with subsequent targeted sequencing identifying *IDH1* and *IDH2* mutations in 4–12% of MDS patients^{23,29,30}. These mutations were discovered to confer a neomorphic function to *IDH1/2* whereby they produce the oncometabolite 2-hydroxyglutarate (2-HG), which appears to directly impair hematopoietic differentiation^{31,32}. 2-HG inhibits a number of epigenetic modifiers including *TET2*³³ and histone demethylases^{33,34}, thereby linking these mutations with the epigenetic machinery.

Analyses of focal deletions on chromosome 7q and 20q led to the discovery of somatic *EZH2*^{35,36} and *ASXL1*³⁷ mutations in 6–12%^{24,35,38} and 14–21%^{37–41} of MDS patients, respectively. *EZH2* is a member of the polycomb repressive complex 2 (PRC2), which is responsible for methylation of lysine 27 on histone 3 (H3K27), which is associated with transcriptional repression. *ASXL1* has been demonstrated to associate with PRC2 and recruit it to target loci, with deletion leading to de-repression of particular genes such as the *HOXA* cluster⁴².

Spliceosome Mutations

In 2011, a whole exome sequencing study demonstrated recurrent mutations of the splicing factor *SF3B1* in 20% of MDS patients⁴³, with a particularly high frequency in disease characterized by ringed sideroblasts (65%). Subsequent whole exome and whole genome sequencing efforts revealed mutations in other splicing factors including *U2AF1*⁴⁴, *ZRSR2*, and *SRSF2*⁴⁵, with splicing pathway mutations being present in 45–85% of all MDS cases. Mutations in the splicing machinery occurred in a mutually exclusive manner, suggesting a common mechanism of action, and mutations appeared to affect recognition of the 3' splice acceptor site on introns, promoting altered pre-mRNA splicing. A mouse model expressing the mutant *Srsf2*^{P95H} allele promoted myelodysplasia and altered the preference of SRSF2 for exonic splicing enhancer motifs, leading to the expression of an altered isoform of *EZH2* that undergoes nonsense mediated decay⁴⁶, in part explaining how altered mRNA splicing may contribute to alterations in hematopoiesis. Similarly, expression of *U2af1*^{S34F} in an inducible transgenic mouse model led to alterations in splicing of RNA processing genes, ribosomal genes, and a number of genes recurrently mutated in MDS and AML⁴⁷.

Other Mutations

While mutations in epigenetic modifiers and splicing factors comprise the most commonly observed recurrent mutations in MDS, many less frequent mutations encompass distinct classes of genes such as *TP53*, transcription factors (*RUNX1*, *BCOR*), kinase signaling (*NRAS/KRAS*, *CBL*, *JAK2*), and cohesion complex members (*STAG2*, *SMC3*, *RAD21*)⁴⁸. Despite being infrequent, such mutations may drive disease biology and clinical phenotypes. For example, a *Runx1* deficient mouse model recapitulating the loss of function mutations seen in MDS was characterized by hematopoietic stem and progenitor cells (HSPCs) with reduced ribosome biogenesis, stress resistance, and a competitive advantage with respect to wild-type HSPCs⁴⁹, recapitulating several hallmarks of MDS.

Refining Prognostic Models with Molecular Genetic Abnormalities

The use of clinical prediction tools such as the International Prognostic Scoring System (IPSS)⁵⁰, developed in 1997, has long allowed clinicians to use parameters such as bone marrow blast percentage and cytogenetic abnormalities to counsel patients and inform treatment decisions. In 2012 a revised IPSS (IPSS-R) scoring system was established, incorporating an expanded set of chromosomal abnormalities and age, as well as measurements of cytopenias and excess blasts weighted based on severity⁵¹.

Although none of the established prognostic scoring systems to date incorporated molecular genetic abnormalities, many prior studies have suggested a potential role for particular gene

mutations in refining prognostic models. For example, prior analyses of *TP53*⁵², *DNMT3A*²², *ASXL1*^{37,39}, *IDH1*⁵³, and *SRSF2*^{54–57} mutations found associations with poor prognosis. However, such analyses largely assessed single genes in isolation and may have incompletely determined the independent contributions of mutated genes in the context of complex genotypes. To address this, Bejar and colleagues sequenced 111 cancer-associated genes in 439 patients with MDS, identifying *ASXL1*, *RUNX1*, *TP53*, *EZH2*, and *ETV6* as independent predictors of poor overall survival in a multivariate analysis controlling for IPSS classification³⁸. The large number of patients surveyed also allowed for genotype/phenotype correlations, such as the association of *RUNX1*, *TP53*, and *NRAS* mutations with severe thrombocytopenia and an elevated blast percentage, and the association of *TP53* mutations with complex karyotypes. Such clinical associations may in part explain how the prognostic impact of such mutations is indirectly captured by the IPSS.

A subsequent study by Papaemmanuil and colleagues performed targeted sequencing of 111 genes across 738 patients with MDS, identifying driver mutations in 43 genes⁴⁸. Of note, 36 of these genes were in a “long tail” of genes mutated in <5% of all patients, but in aggregate these more rare mutations comprised 33.5% of all mutations, underscoring the significant mutational heterogeneity of MDS. While this study corroborated the poor prognostic import of genes such as *ASXL1*, *RUNX1*, and *TP53*, as well as *SRSF2* and *U2AF1* (not included in the study by Bejar and colleagues), it also identified a correlation between the number of driver mutations present and decreased leukemia-free survival. Whether or not the detected mutations were clonal or subclonal did not impact on their prognostic import, suggesting that use of sensitive sequencing techniques to identify small subclones will be needed to maximize the clinical potential of identifying these mutations. However, utilizing a clinical data set incorporating standard clinical variables, the addition of molecular genetic data achieved only a marginal non-significant increase in prognostic value. Given the “long tail” of infrequently mutated genes in MDS, further studies in larger cohorts of MDS patients (such as the ongoing IPSS-R Mol.) may be necessary to derive robust prognostic schema utilizing molecular genetics. However, at the current time the extent to which molecular genetics can improve on existing clinical risk-stratification schema remains unresolved, which is in part a testament to the robustness of prognostic algorithms developed by astute clinicians.

The Contribution of Molecular Genetic Abnormalities to Understanding the Origin and Clonal Evolution of MDS

The finding of MDS associated cytogenetic abnormalities in hematopoietic stem cells (HSCs) purified from MDS patient bone marrows has long suggested that MDS is initiated in this primitive cell population^{58–60}. More recent studies have demonstrated that essentially all recurrent somatic mutations observed in low-risk MDS are acquired in HSCs⁶¹. This stands in contrast to AML, in which the full complement of mutations is not acquired in HSCs, but rather a more committed myeloid progenitor, with a subset of mutations found in functionally normal “pre-leukemic” HSCs^{26,27} (Figure 1). Many mutations found in pre-leukemic HSCs are also recurrently mutated in MDS (e.g. *DNMT3a*, *TET2*, and *ASXL1*), and when MDS progresses to AML, additional mutations are likely acquired in MDS progenitors^{61,62}. A critical question then is what distinguishes a normally functioning “pre-

leukemic” HSC from an MDS HSC that gives rise to ineffective hematopoiesis. The fact that many healthy older adults may exhibit clonal hematopoiesis with MDS associated mutations such as those in *DNMT3A*, *TET2*, and *ASXL1*^{63,64} suggests that more than one mutation is required to produce MDS. Analysis of the allelic frequencies of mutations in large sequencing datasets⁴⁸ is beginning to shed light on the order of acquisition of mutations in MDS, but further functional studies using animal models will be necessary to determine the minimal complement of mutations necessary to give rise to MDS. Targeting MDS stem cells that harbor this minimal complement of mutations will need to be a feature of any novel therapies capable of inducing durable disease remissions. The order of acquisition of mutations itself may have prognostic significance, as has recently been demonstrated for myeloproliferative neoplasms harboring both *TET2* and *JAK2* mutations⁶⁵.

Diagnostic Utility of Molecular Genetics

The diagnosis of MDS remains challenging, particularly in cases without obvious morphologic dysplasia, excess blasts, or abnormal cytogenetics. This has led to the development of terms such as idiopathic cytopenias of unknown significance (ICUS)⁶⁶. Molecular genetic abnormalities may provide evidence for clonal hematopoiesis and allow for reclassification of some cases of ICUS to low-risk MDS, but designating all patients with ICUS and an MDS associated mutation inappropriately presumes causality and will almost certainly lead to overdiagnosis. Since multiple mutations are likely to be necessary to develop MDS, more comprehensive sequencing of patients with ICUS with prospective clinical follow-up will be necessary to establish what combinations of mutations are specific for fully penetrant disease.

Selecting Patients for Transplantation

Although allogeneic transplantation is the only potentially curative therapeutic modality for MDS, its potential morbidity and mortality need to be balanced with an assessment of disease risk. Indeed, a Markov-decision analysis of a large MDS transplant registry suggested that transplantation of patients with lower-risk disease may lead to worse outcomes⁶⁷. Molecular genetics may help to identify patients with lower-risk MDS that actually have higher disease risk and who may benefit from early transplantation. To identify such a subgroup, Bejar and colleagues sequenced 22 genes in 288 patients with lower-risk MDS, finding *EZH2*, *RUNX1*, *TP53*, and *ASXL1* to be associated with worse outcomes²⁴. However, in a multivariate analysis incorporating all other clinical data, only *EZH2* mutations retained independent prognostic significance. Thus, similar to the study by Papaemmanuil and colleagues discussed above, the marginal contribution of molecular genetic data to prognostication independent of other clinical data remains limited. Ultimately, the most relevant question is whether allogeneic transplantation can modify the outcome of poor-risk molecular genetics, as has been demonstrated for some AML genotypes⁶⁸. Particular mutations such as those in *TP53* and *TET2* predict for such a poor outcome even after allogeneic transplantation⁶⁹, that the value of transplantation in such patients remains questionable. Such patients might be targeted for novel non-transplant therapies, intensified conditioning regimens, and/or post-transplant prophylaxis strategies. An examination of molecular genetics in prospective trials, such as an ongoing CIBMTR trial comparing outcomes with allogeneic transplant versus hypomethylating agent therapy

or best supportive care, will be necessary to fully assess the ability of transplantation to overcome the poor-risk conferred by high-risk molecular genetic abnormalities.

Predicting Disease Response to Conventional Therapies

Mutations in *TET2* and *DNMT3A* may predict for an increased likelihood of response to hypomethylating agents^{70–72}, but this predictive value is modest, does not correlate with improved survival, and does not influence clinical management in the absence of an alternative first-line therapy for MDS. Moreover, a recent study examining the response of patients with CMML to decitabine was unable to confirm these associations, but rather found that DNA methylation profiles at diagnosis may be highly predictive of response⁷³. For patients with isolated del(5q) who respond exceptionally well to lenalidomide⁷⁴, the presence of *TP53* mutations predict for lower response rates and a higher rate of progression to AML^{75,76}. Again, given the absence of alternative therapies, the presence of these mutations does not currently influence clinical management.

Novel Therapies Targeting Molecular Genetic Abnormalities

Ongoing clinical trials utilizing innovative new therapies for high-risk MDS include the use of agents such as histone deacetylase inhibitors⁷⁷, hypomethylating agents with improved pharmacokinetic properties such as SGI110⁷⁸, and inhibitors of the phosphoinositide 3-kinase and polo-like kinase pathways such as Rigosertib^{79–81}. For lower risk MDS, novel erythropoietic agents that inhibit members of the TGF- β family such as GDF11 are being investigated (e.g. LY-2157299⁸², Sotatercept⁸³, and ACE-536⁸⁴). GDF11 appears to be overexpressed in MDS and is also associated with aging⁸⁵, but it is unclear if it is enriched in any particular MDS subtype. As such, these therapies, along with almost all other novel therapies being investigated, are not targeted towards any specific molecular genetic subgroup. One exception is the use of IDH1/2 inhibitors in high risk MDS patients with IDH mutations, which have demonstrated impressive response rates as single agents⁸⁶. Inhibitors of the splicing machinery are currently in early phase trials for CLL^{87,88} and appear to have selective toxicity to MDS cells in pre-clinical models^{89–91}—they represent a promising therapy that might benefit a significant number of patients given the prevalence of splicing mutations in MDS. Comprehensive sequencing of patients treated with novel therapies not targeted towards any particular subgroup may help to identify molecular genetic subgroups most likely to benefit from such therapies and lend insight into disease biology and therapeutic mechanisms. An instructive example is the longstanding observation of robust clinical responses to lenalidomide in patients with del(5q) associated MDS⁷⁴, which recently led to significant insights into how lenalidomide modulates the degradation of a haploinsufficient gene on 5q (*CSNK1A1*) to deplete MDS cells in a synthetic lethal manner⁹², revealing a novel therapeutic paradigm that may be applied to many other cancers.

Conclusions

The past decade has witnessed significant advances in our understanding of the molecular underpinnings of MDS, helping to explain its heterogeneous behavior and clinical presentation. However, already robust existing clinical prediction tools and a meager

therapeutic armamentarium limit the utility of molecular genetic abnormalities in predicting prognosis and influencing therapeutic decisions (summarized in Table 1). Perhaps an area of most immediate impact may be the ability of molecular genetics to identify patients with clinically low-risk disease who may have a higher risk for disease progression. Whether allogeneic transplantation can overcome the high-risk conferred by various molecular genetic abnormalities will need to be addressed in ongoing prospective trials. We will undoubtedly continue to discover new molecular genetic abnormalities and potential “druggable” targets through ongoing sequencing efforts. Together, these advances promise to bring our now vast knowledge of the molecular genetics of MDS to bear on clinical decision-making and novel therapeutics.

Acknowledgments

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order of mutational acquisition had a significant influence on disease phenotypes. Patients who acquired *JAK2* mutations first had a higher likelihood of having polycythemia vera and developing thrombosis. By extension, one may expect that the order of acquisition of MDS associated mutations may influence disease phenotypes and outcomes. [PubMed: 25671252]

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Key Points

- Comprehensive sequencing efforts have identified a variety of molecular genetic abnormalities in MDS that in part explain the heterogeneous biology and clinical behavior of these disorders.
- Molecular genetic abnormalities in MDS may be predictive of disease phenotypes, including prognosis and response to therapy, but their utility in the clinic is limited given already robust clinical prognostic algorithms and few effective therapies for MDS.
- Particular molecular genetic abnormalities themselves represent promising therapeutic targets, with a number of ongoing pre-clinical and clinical studies testing novel compounds that may target these abnormalities.

Figure 1. Model of Acquisition of Molecular Genetic Abnormalities in MDS, de novo AML, and Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Many MDS-associated mutations are also seen in CHIP and pre-leukemic hematopoietic stem cells (HSCs), although in CHIP they are much more likely to be sole abnormalities. However, the minimal complement of mutations to give rise to the dysplastic hematopoiesis that characterizes MDS remains unresolved. At the time of leukemia development or leukemic progression of MDS, additional mutations are acquired in myeloid progenitors, which acquire aberrant self renewal (denoted by curved arrow) and become leukemic stem cells.

Table 1

Recurrently Mutated Genes in the Myelodysplastic Syndromes With the Potential to Influence Prognosis and/or Therapeutic Decisions

Gene	Frequency	Prognostic Significance	Phenotype Correlation	Therapeutic/Diagnostic Implications	Target of Novel Therapy?	References
<i>DNMT3A</i>	3–13%	Poor in some small studies, but no effect on OS in larger multivariate analyses.	None	Conflicting studies on whether it may predict response to hypomethylating agents. May be present in many healthy older adults.	No	21–24,72,73
<i>TET2</i>	19–26%	No effect on OS in the largest studies. May predict for a particularly poor outcome after alloHSCT	None	Conflicting studies on whether it may predict response to hypomethylating agents. Given poor outcomes after alloHSCT, may need to target for novel therapies to replace or improve on transplant. May be present in many healthy older adults.	No	12–15,70,72,73
<i>ASXL1</i>	14–21%	Poor in multivariate analyses accounting for other mutations and clinical variables	None	These high risk patients should be considered for alloHSCT. May be present in many healthy older adults.	No	37–41
<i>EZH2</i>	6–12%	Poor in multivariate analyses accounting for other mutations and clinical variables.	None	These high risk patients should be considered for alloHSCT. In particular, <i>EZH2</i> mutations may independently predict for a worse prognosis in patients otherwise low-risk by clinical variables.	No	24,35,38
<i>SRSF2</i>	1.5%–11.6%	Poor in multivariate analyses accounting for other mutations and clinical variables	Enriched in CMML	These high risk patients should be considered for alloHSCT.	Yes (Spliceosome inhibitors)	45,48,90,93
<i>SP3B1</i>	20%	Favorable, with an indolent disease course. Less likely to have mutations in other genes associated with a poor prognosis	High association with ringed-sideroblasts	The patients tend to have a very indolent disease course. These mutations are also found in other malignancies, such as CLL.	Yes (Spliceosome inhibitors)	24,43,48,90,94–96
<i>U2AF1</i>	8–12%	Poor in multivariate analyses accounting for other mutations and clinical variables. High rate of co-occurrence with <i>ASXL1</i> and <i>del(20q)</i> .	Increased risk of progression to AML	These high risk patients should be considered for alloHSCT.	Yes (Spliceosome inhibitors)	44,45,48,90
<i>IDH1/2</i>	4–12%	Possible poor prognostic impact of <i>IDH/R132</i> and <i>IDH/R172</i> mutations (but not <i>IDH/R140Q</i>). Not substantiated in large multivariate analyses accounting for other mutations.	None	Should prioritize patients with these mutations and high-risk MDS for IDH inhibitor trials.	Yes (IDH1/2 inhibitors)	38,48,53,86
<i>TP53</i>	5%	Poor in multivariate analyses accounting for other mutations and clinical variables. High rate of co-occurrence with complex karyotypes and fewer cooperating lesions.	Associated with excess blasts and thrombocytopenia.	Given poor outcomes after alloHSCT, may need to target for novel therapies to replace or improve on transplant. In the context of <i>del(5q)</i> , predicts for less frequent cytogenetic responses to lenalidomide and a higher rate of progression to AML.	No	38,48,75,76,97,98