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The spectrum of molecular aberrations in myelodysplastic syndromes: in the shadow of acute myeloid leukemia

David P. Steensma

Division of Hematology, Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA.
E-mail: Steensma.david@mayo.edu

How are the genetic profiles of acute myeloid leukemia (AML) and the myelodysplastic syndromes (MDS) similar, and how do these two disorders differ? These are complex questions – and difficult ones to answer intelligently right now, chiefly because so little is currently understood about the molecular etiology of MDS. Furthermore, despite several features in common, both AML and MDS are phenotypically and genetically heterogeneous, making it hard to tease out unifying threads binding their molecular pathobiology. Yet some recent progress has been made, including insights from a large mutation screening study published in this issue of Haematologica. A few common themes are beginning to emerge, and the near future holds great promise (Figure 1).

Molecular basis of AML

There is widespread support for the concept that the development of AML requires at least two somatic gene alterations: one mutation to augment the rate of cellular proliferation or enhance cell survival (a *class I mutation*, usually constitutively activating a tyrosine kinase or a RAS family member), and another mutation impairing normal cellular differentiation (a *class II mutation*, usually deregulating a hematopoietic transcription factor or a transcriptional co-activator, such as homeobox family members or the components of core binding factor).[1] The repertoire of known genetic changes capable of filling one of those two pathologic demands continues to expand. Recurrent chromosomal translocations, gene rearrangements (including amplifications and deletions), and point mutations are all now well recognized as important contributors to the myeloid neoplastic phenotype.[2]

Recently described mutations in the nuclear-cytoplasmic shuttling factor nucleophosmin (NPM1) lay claim to the title of the overall most frequent AML-associated genetic lesion, common balanced translocations notwithstanding.[3] Truncating NPM1 mutations result in protein mislocalization and are remarkably prevalent in AML, being detectable in 45-50% of cases of *de novo* disease with a normal karyotype and in 5-10% of patients with abnormal cytogenetics.[3,4] More generally, the likelihood of finding a given mutation such as NPM1 depends on the karyotype, the clinicopathological subtype of AML, and whether or not the patient has a history of therapy with DNA-damaging agents (*therapy-related AML*, t-AML) or was diagnosed with another clonal myeloid disorder prior to developing AML (*secondary AML*, s-AML).

NPM1 mutations are followed in frequency by the former *record holder*: activating mutations of the *FLT3* tyrosine kinase, which often co-exist with NPM1 mutations and likely co-operate in leukemogenesis.[5] Internal tandem duplications of the juxtamembrane domain of *FLT3* can be found in ~20-25% of patients with *de novo* AML, and, like NPM1 mutations, are more common in people with a normal karyotype, while FLT3 D835 activating loop point mutations are detectable in another 5-10% of cases.[6]

Over the last two decades, careful work by dozens of research groups has defined the prognostic implications of recurrent AML-associated karyotypes (not considered further here), and also uncovered a number of other important acquired mutations in AML (e.g., TP53, NRAS, KRAS, KIT, PTPN11, CEBPA, CSF1R (C-FMS), NF1, and BRAF point mutations, and MLL partial tandem duplications).[1] However, each of those individual

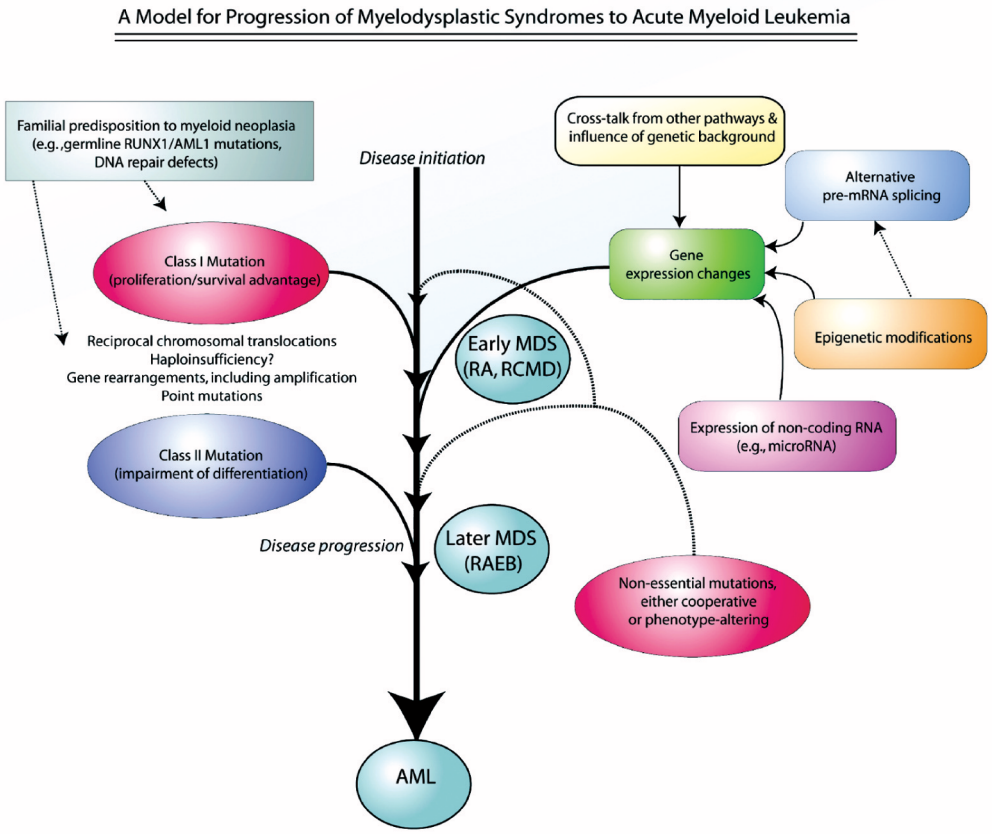

Figure 1. A general model for the development and progression of MDS and AML. Patients may come to clinical attention at any point along the Early MDS-Advanced MDS-AML spectrum; antecedent MDS is, of course, not necessary for the development of AML. Myeloid neoplasia is uncommonly familial, but some patients are predisposed to the development of MDS or AML by inborn defects in DNA repair or checkpoint control/mutation surveillance, or, rarely, by germline mutations in hematopoietic transcription factors such as RUNX1/AML1 (mutated in *familial platelet disorder with predisposition to AML*.) The patient's idiosyncratic genetic background influences the risk of developing MDS/AML, and may also modify the phenotype once disease has developed. Acquirement of somatic *class I and II* mutations by any mechanism (e.g. chromosomal rearrangements such as reciprocal translocations, point mutations), are key events in the development of disease, and are frequently environmentally mediated by exposure to DNA-damaging agents. Additional mutations may contribute to disease progression without being strictly necessary for the process (e.g., TP53 mutations), or may modify the phenotype without affecting the natural history of disease (e.g., somatic ATRX mutations that cause acquired alpha thalassemia in MDS). Finally, gene expression changes in the absence of alterations in the coding DNA sequence have a major influence on disease phenotype and progression. These transcript expression changes can be mediated by several mechanisms, including alternative RNA splicing, epigenetic changes (e.g., histone acetylation, CpG methylation, chromatin remodeling – which may influence other processes, including splicing), expression of non-coding RNA, mutations in gene regulatory elements, and cross-talk from other signaling pathways. The temporal order of events is not necessarily as depicted.

point mutations is present in <10% of AML cases. This observation underscores both the redundancy of cell biology and the complexity of neoplastic transformation – as well as highlighting the daunting challenge of designing targeted *boutique* therapies tailored to the molecular idiosyncrasies in each patient with AML.

Molecular basis of MDS

Unfortunately, our understanding of the molecular pathology of MDS remains considerably more primitive than our understanding of AML disease biology.[7,8] Recurrent cytogenetic abnormalities are well described in MDS, but chromosomal gains and losses predominate; informative reciprocal translocations such as those that led to the first discoveries in AML genetics are rare in MDS. The point mutations detected in AML analyses have also turned out to be uncommon in MDS, and the few mutations first described in MDS, such as deletions in mitochondrial DNA, have proven to be the exception rather than the rule.[7,9]

Each of the gross chromosomal abnormalities seen with the highest frequency in MDS patients – loss of material from chromosomes 5, 7, 13, 20, and the sex chromosomes, or trisomy 8 – remains mechanistically obscure. Only a smattering of uncommon MDS-associated rearrangements have been further characterized, such as those involving 3q26 (corresponding to the zinc-finger DNA-binding protein MDS1-EVI1), 3q25.1 (the p53-regulator MLF1), or 1p36 (PRDM16, formerly MEL1, another zinc-finger transcription factor).[10] Notably, all three of these latter genes are involved in balanced translocations, which are observed in fewer than 5% of MDS patients.

Despite these limitations, chromosomal rearrange-

ments in MDS detectable by conventional G-banded karyotyping continue to be clinically important. An abnormal karyotype can prove clonality, securing what might have been a tenuous morphologic diagnosis;[11] several karyotypes are prognostically useful;[12] and, in the case of deletions of chromosome 5q, knowledge of the karyotype can even help in selecting a specific treatment, lenalidomide.[13] However, we can not escape the sad truth that our knowledge of MDS-associated karyotypes has thus far yielded precious few solid molecular insights, hampering development of targeted therapies like those currently undergoing clinical trials in AML. Twenty years ago, three promising papers concerning MDS-associated point mutations appeared in *Nature*: descriptions of NRAS and KRAS codon 13 mutations, and evidence for the lack of HRAS mutations.[14-16] Investigators hoped that MDS would quickly yield to the new tools of molecular biology, but that august scientific journal has not yet received any other MDS-related manuscripts worth publishing. Connecting the dots between recurrent cytogenetic findings in MDS and specific molecular genetic lesions proved more difficult than initially imagined.

Genetic pathways to MDS and AML

In this issue of Haematologica, Bacher and her colleagues in Germany compare the prevalence of five of the *usual suspect* point mutations – molecular abnormalities commonly associated with AML – in a large cohort of patients with AML (n=4130) and a smaller but still sizeable group suffering from MDS (n=381, primarily patients with refractory anemia with excess blasts, RAEB).[17] The investigators included four groups of AML patients in their analysis: apparently *de novo* disease, t-AML, s-AML, and relapsed AML – entities that may have distinct molecular pathways – in order to more clearly define when in leukemogenesis these mutations arise.

Collectively, Bacher and her colleagues observed the highest rate of mutations in relapsed AML, presumably because such patients have been most heavily exposed to DNA damaging agents and have had the most opportunity to accumulate molecular injuries. The observed mutation rate was also higher in s-AML than in MDS, confirming that AML arising from MDS is more likely to have one of the few mutations we know about, and that their acquisition can be a key transformational event along the MDS-AML continuum.

While mutations were common in all of the AML cohorts, none of the five mutations examined was present in more than 6.3% of MDS cases (NRAS mutations were the most common of the five). The German investigators also found vanishingly few mutations in MDS cases without excess blasts: only two mutations in 49 patients with refractory anemia or refractory anemia with ringed sideroblasts, and three mutations in 22 patients with chronic myelomonocytic leukemia. This finding emphasizes that more work is needed with respect to initiating events, in particular in MDS. Almost all of the mutations were detected in patients with RAEB, in which the increased blast proportion signifies that the marrow is already well along the pathway to overt AML.

Early MDS differs from more advanced MDS in terms of the balance between cell proliferation and intramedullary apoptosis.[18,19] In addition, while proliferation is increased above physiological levels in all MDS subtypes, a modicum of normal hematopoietic maturation is retained (albeit grossly disordered) early on, until a poorly differentiated leukemic clone evolves and begins its domination. At that point, it is perhaps not surprising that leukemia-like mutations can be detected more regularly. But it is still unclear what was going on earlier in the disease course.

Bacher and her collaborators did not study the prevalence of *NPM1* mutations, which others have shown are almost non-existent in MDS,[20] nor did they examine the most common MDS-associated molecular genetic abnormality described to date, point mutations (often biallelic) in the Runt domain of the transcription factor AML1/RUNX1.[21] In AML, AML1/RUNX1 point mutations do occur, but are less common than translocations such as t(8;21) [AML1-ETO], which is present in about 30% of AML-M2. The opposite is true in MDS: translocations involving AML1/RUNX1 are rare, but point mutations are surprisingly common, present in 7-25% of cases, especially t-MDS and cases with monosomy 7.

Informing projects such as that of Bacher and colleagues is the work of Pedersen-Bjergaard and his coworkers in Copenhagen, who have beautifully outlined eight different genetic pathways underlying t-MDS and t-AML – disorders that are of special interest because they provide the clearest view of sequential acquisition of mutations as myeloid neoplasia evolves and progresses.[22,23] Most recently, this Danish group further characterized their eight pathways by looking at the prevalence of common point mutations similar to those studied by Bacher and colleagues, including AML1/RUNX1, RAS, TP53, BRAF, CKIT, FLT3, MLL, and JAK2.[22] Although the number of patients examined was relatively small (n=140), several insights were gained. These included a better appreciation of the high frequency of TP53 mutations in patients with t-MDS/t-AML and chromosome 5 abnormalities (74%), as well as further evidence for the mutual exclusiveness of mutations within each class (I and II).

This is, however, clearly work in progress. Many patients with both t-MDS and t-AML have only a minimally informative karyotypic abnormality (e.g. monosomy 7) without a detectable molecular genetic lesion, while others have a normal karyotype without a known point mutation or have atypical or unique

lesions of uncertain general significance. Another major challenge is to define t-AML/t-MDS clearly: some patients who have received alkylating agents or topoisomerase II inhibitors may still develop *de novo* MDS/AML, whereas some people presenting with apparently *de novo* MDS or AML likely have been unwittingly exposed to DNA-damaging agents.

Prospects for the future

What are the prospects for further clarification of the MDS molecular profile in the next several years? Recent exciting developments with respect to the myeloproliferative disorders (MPD) may be cause for hope.

In MPD, as in early MDS, impairment of cell differentiation is not strictly necessary. But in MPD, a class I mutation that gives a hematopoietic clone a proliferative and/or survival advantage appears to be an absolute requirement for development of disease (this is clearer in MPD than it is in early MDS). The BCR-ABL fusion kinase provides chronic myeloid leukemia cells with just such an advantage. BCR-ABL was recognized decades ago, but most cases of Philadelphia chromosome-negative MPD remained etiologically mysterious until recently.

Discovery of the JAK2 V617F mutation in early 2005,[24] followed by elucidation of JAK2 exon 12 mutations in 2007,[25] filled in a large blank spot on the MPD map.[26] Essentially all patients with polycythemia vera have an acquired JAK2 mutation, as do about half of those with essential thrombocythemia or idiopathic myelofibrosis and roughly 20% of atypical MPD cases.[27] The availability of JAK2 mutation testing has been extremely useful diagnostically, and clinical trials of JAK2-targeted drugs are about to begin.[26] In view of the phenotypic heterogeneity of the MPD now linked by a common mutation (one clearly pathologically relevant, even if it is not truly the initiating event), it seems fair to ask: will there ever be a comparable highly prevalent biomarker for MDS – a *JAK2 equivalent*?

Despite our poor track record of defining common MDS-associated molecular lesions, I believe that optimism is indicated. In view of the relentless ongoing technical advances in the tools for genomic discovery, coupled with the declining cost of large-scale sequencing, it seems unlikely that MDS can hold its secrets for much longer. Admittedly, new discoveries have been slow in coming from the once-hyped technologies such as oligonucleotide cDNA expression microarrays, the first genome-wide screening tool. RNA expression changes are clearly important in myeloid neoplasia (e.g., BAALC expression and the ratio of *RUNX3/ATRX* expression are prognostic in *de novo* AML),[28,29] so it would be premature to abandon this line of investigation in MDS. Yet initial reports of cDNA microarray studies in MDS were reported more than 5 years ago,[30,31] and translating these observed RNA expression patterns into better biological understanding of the disease has proven to be an uphill battle. Instead, what is so exciting currently is that the sheer range of array platforms for global analyses has greatly expanded, allowing the problem of MDS to be approached from many angles and therefore increasing the likelihood of an important discovery.

Laboratories now have access to array-based comparative genomic hybridization (CGH) tools; chips with tens of thousands of probes for single nucleotide polymorphisms, allowing a genome-wide hunt for loss of heterozygosity and uniparental disomy; platforms for assaying non-coding RNA molecules, including trendy microRNA; exon-based arrays to look for neoplasia-associated patterns of alternative pre-mRNA splicing; resequencing arrays for large-scale mutation detection efforts; and even *ChIP-on-chip*, which helps extend our vision beyond classical genetics into the exciting world of epigenetics. Array CGH seems particularly promising: unlike RNA expression, which can go up or down for many reasons not necessarily directly connected to a disease process, recurrent alterations at the DNA level are often meaningful, even when they are not central pathobiological events. Just as important as the expanding range of biotechnological tools are recent developments in bioinformatics, which improve our ability to understand the patterns detected and to design follow-up experiments.

These prospects are inspiring young investigators, drawing them to our field in greater numbers. We can only hope that research funding improves to allow the good work to continue, and that this enthusiasm translates into improved outcomes for our patients with MDS in the near future.

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