Myelodysplastic Syndromes

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Molecular Pathogenesis of MDS

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Clonal disorders of hematopoiesis, such as myelodysplastic syndromes (MDS) and myeloproliferative diseases (MPD), affect both hematopoietic stem cells and progenitor cells within the erythroid, platelet and granulocytic lineages and can have devastating consequences in children and adults. The genetic features of these diseases often include clonal, nonrandom chromosomal deletions (e.g., 7q–, 5q–, 20q–, 6q–, 11q– and 13q–) that appear to inactivate tumor suppressor genes required for the normal development of myeloid cells (reviewed in Bench[1] and Fenaux[2]). These putative tumor suppressors have proved to be much more difficult to identify than oncogenes activated by chromosomal translocations, the other major class of chromosomal lesions in MDS and MPD.[3] Although MDS and MPD are almost certainly caused by mutations in stem/progenitor cells,[4] the role of inactivated tumor suppressor genes in this process remains poorly understood. In a small portion of myeloid diseases, mutations have been identified in genes encoding factors known to be required for normal hematopoiesis, such as *PU.1*, *RUNX1*, *CTNNA1* (α-catenin) and *c/EBPα*, and implicating these genes as tumor suppressors.[5-7] Nonetheless, the identities of most deletion-associated tumor suppressors in these diseases remains elusive, despite complete sequencing of the human genome. The deleted regions detected by cytogenetic methods are generally very large, containing many hundreds of genes, thus making it hard to locate the critical affected gene or genes. It is also unclear whether dysfunctional myelopoiesis results from haploinsufficiency, associated with the deletion of one allele, or from homozygous inactivation due to additional point mutations or microdeletions of the retained wild-type allele. In general MDS have proved surprisingly resistant to conventional treatments. Targeted therapeutic advances in MDS will likely depend on a full comprehension of underlying molecular mechanisms, in particular the tumor suppressor genes lost through clonal, nonrandom chromosomal deletions, such as the 7q– and (del)5q.

Myelodysplastic syndrome (MDS) refers to a group of clonal disorders characterized by trilineage defects in hematopoiesis, including the erythrocytic, granulocytic, and megakaryocytic lineages. Although clonal, it is sometimes considered a premalignant condition that often progresses to acute myeloid leukemia (AML), when additional genetic abnormalities are acquired.[8-10] Overall, MDS affects approximately 1 in 500 persons over 60 years of age, making it the most common hematologic malignancy in this age group;[11] it may develop at any age, including childhood. As a complication associated with aggressive treatment of other cancers, MDS shows a high correlation with exposure to radiation, alkylating agents or topoisomerase II inhibitors.[12-17] MDS often develops following autologous bone marrow transplantation, affecting 20% of patients with non-Hodgkin lymphomas who received bone marrow transplants.[13,15,18,19] The prognosis for patients with primary or secondary MDS remains poor, especially in the elderly. MDS usually requires allogeneic bone marrow transplantation for permanent cure, but unfortunately, older patients cannot generally tolerate this procedure, leaving them without effective alternatives. In a study of adult patients with primary MDS, only 6% were alive and in remission 7 years after diagnosis.[20] Children with MDS who undergo bone marrow transplantation have a 58% survival rate after 3 years, as compared with an average survival of only 0.9 years for those who do not receive a transplant.[21] These bleak statistics underscore the importance of understand-

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ing MDS at the molecular level in order to expand the repertoire of biologically based therapies. Improvement in the treatment of acute promyelocytic leukemia (M3 subtype), using all-*trans*-retinoic acid to induce differentiation of malignant promyeloblasts, illustrates the potential of such strategies.[22-24]

Although MDS has been recognized as an important disease for more than 50 years, its molecular pathogenesis and the molecular basis for its progression to AML remain largely undefined. A model of MDS molecular pathogenesis has been proposed whereby a normal hematopoietic stem cell acquires successive genetic abnormalities that ultimately lead to malignant transformation and clonal expansion. Evidence for clonality in MDS comes primarily from nonrandom X-inactivation studies performed on the bone marrow cells of female patients with MDS. These studies demonstrate clonal involvement of hematopoietic cells in this disorder. Early mutations in stem cells may cause differentiation arrest leading to dysplasia, whereas subsequent defects affecting myeloid cell proliferation may cause the clonal expansion of aberrant cells and frank AML. Although many chromosomal abnormalities have been detected in MDS (e.g., 5q– and monosomy 7), the genes involved are yet to be identified, and it is unknown whether these genetic aberrations are initial events leading to the development of MDS or are secondary events.

Myeloproliferative disorders (MPD), such as chronic myeloid leukemia (CML) and myeloid metaplasia with myelofibrosis (MMM), are hematopoietic stem cell diseases characterized by uncontrolled growth of granulocytes and other hematopoietic cells, resulting in clonal expansion of those lineages. A role for mutationally activated tyrosine kinase genes has now been established in most cases with MPD (**Figure 1**; see Color Figures, page 552). Recently, following earlier discoveries of mutant tyrosine kinases in CML, chronic myelomonocytic leukemia, hypereosinophilic syndrome and systemic mast cell disease, four groups reported a specific activating mutation in the tyrosine kinase JAK2 in three distinct forms of MPD—polycythemia rubra vera (PRV), essential thrombocythemia (ET), and MMM.[4,25-27] Despite these advances in the identification of tyrosine kinase oncogenes, it has been difficult to identify the tumor suppressors whose inactivation contributes to the pathogenesis of MPD and the chromosomal regions of deletion are often shared between MDS and MPD (**Figure 1**; see Color Figures, page 552). Patients with neurofibromatosis type I, with inactivation of the neurofibromin 1 (NF1) tumor-suppressor gene, frequently develop juvenile myelomonocytic leukemia (JMML); however, the onset of this and other types of MPD is commonly associated with complex karyotypes indicating the involvement of additional genetic pathways in the pathogenesis of MPD.[28] Clearly, a major advance in the fields of MDS and MPD will be made by the identification of additional mutations involved in the development and progression of these families of diseases.

Chromosomal Loss and Malignant Progression in Human Myeloid Diseases

Based on cytogenetic findings, MDS and AML can be broadly subdivided into cases with (i) normal karyotypes, (ii) balanced chromosomal aberrations leading to the generation of fusion oncogenes and (iii) complex karyotypes (more than 3 chromosomal aberrations). Complex chromosomal aberrations (CCAs) are associated with the most unfavorable prognosis among subtypes of MDS and AML, and MDS cases with a complex karyotype have a high propensity to evolve to AML. Despite intensive treatment including allogeneic stem cell transplantation, long-term remissions are achieved in less than 10% of patients with CCAs.[29] The frequency of CCAs is remarkably high: 20% of de novo AMLs, 30% of de novo MDSs, 24% of secondary AMLs and up to 50% of therapy-related AML and MDS cases.[30,31] The lower frequency of CCAs in de novo AML reflects the higher prevalence of classic translocation-generated oncogenes (e.g., *AML1-ETO*, *PML-RARα* and many others) in this disease compared with their paucity in MDS.[3] Thus, myeloid leukemias and myelodysplastic syndromes with CCAs constitute important clinical entities in need of improved therapeutic strategies.

Cytogenetic studies have revealed both balanced chromosomal abnormalities leading to the generation of fusion oncogenes and unbalanced recurrent aberrations, most commonly –5, 5q–, –7, 7q–, +8, 11q–, 13q– and 20q–,[1,2] suggesting that genes within these regions have a role in MDS/MPD pathogenesis (**Figure 1**; see Color Figures, page 552). Finding the genes affected by such deletions poses a major investigative challenge, but will be necessary to accelerate progress in research and treatment of these myeloid diseases.

Clonal chromosomal abnormalities are observed in bone marrow cells from 30% to 50% of de novo MDS cases and 80% of secondary MDS patients. The predominant abnormalities discovered in MDS are nonrandom chromosomal deletions, suggesting a pathogenic mechanism based on loss of tumor suppressor genes or haploinsufficiency of genes necessary for normal myelopoiesis. Common cytogenetic abnormalities in MDS include loss of chromosome 7 or partial deletions of chromosome arms 5q, 20q, 11q, or 7q. In addition, juvenile chronic myelomonocytic leukemia often involves monosomy 7, together with mutations of the NF1 gene.[28] In a study of 1663 cases of MDS, 1098 (66%) had a single chromosomal abnormality, 237 (22%) were monosomic for chromosome 7, and 431 (39%) had a partial deletion of chromosome 5. Other abnormalities included chromosomes 6, 9, 11, 12, 13, and 17. Importantly, most of these genetic abnormalities correlate with prognosis. After intensive chemotherapy, 60% of patients with an apparently normal karyotype entered complete remission (average duration, 16 months), while patients with chromosome 5 or 7 deletions or complex chromosomal abnormalities had a 20% remission rate (average duration, 4 to 5 months). Similarly, secondary MDS usually displays monosomies of chromosome 5 or 7 or partial deletions involving

5q or 7q, with chromosome 7 defects associated with decreased survival time.[13]

Although the majority of putative tumor suppressors in MDS have not been cloned, many chromosomal translocation-mediated oncogenes (see **Figure 1** [in Color Figures, page 552] reviewed in Look[3]) and a few of the tumor suppressors have been identified. For example, genes inactivated in MDS comprise a relatively small number of cases and include *P53, RB, WT-1, NF1, AML1, C/EBPα*, α-catenin (*CTNNA1*) and nucleophosmin (*NPM*) (reviewed in Tenen[6] and Side[28]). However, only two of these genes (*P53* and *CTNNA1)* lie within the clinically prominent chromosomal deletions in MDS or MPD, suggesting that many of the principal tumor suppressors responsible for these myeloid diseases have yet to be identified.

MLL Amplification in MDS

In contrast to AMLs harboring oncogenic transcription factor fusions, hardly any oncogene activation has been assigned specifically to MDS and AML with CCAs. One exception is the 11q23 region and its resident MLL gene, which is amplified in a significant fraction of MDS and AML with karyotypic complexity and an adverse prognosis.[32-35] MLL has long been recognized as an important component of translocation-generated fusion proteins. In contrast to other oncogenic fusion proteins, MLL participates in translocations with more than 40 different partner chromosomal loci. Homodimerization of the chimeric proteins appears to underlie the promiscuity of MLL in its ability to combine with many fusion partners, at least for a subset of its productive fusions.[36]

The new studies mentioned above suggested that amplification of MLL represents a new mechanism of oncogenic activation of this gene. A recent study confirmed the importance of MLL within the 11q23 amplicon by analysis of MLL target genes like HOXA9 and MEIS1.[37] It has long been recognized that HOXA9 is one of the important target genes of MLL and recent reports from several independent laboratories, including ours, provide a comprehensive view of other HOX genes that may be inappropriately activated in leukemias with MLL rearrangements.[38,39] Very recently, Hoxa7 and Hoxa9 were shown to be essential for MLL-dependent leukemogenesis in vivo.[40]

Thus, aberrant MLL activation in MDS/AML and consequent dysregulation of its downstream targets are of clinical importance. Indeed, the remarkable synergy between MLL gene amplification and loss of 5q in MDS and AML are reported to result in an extremely poor overall survival rate of 30 days.[35] Moreover, these data provide an important clue regarding the mechanism of disease evolution. It should be stressed that the impact of dysregulated MLL and HOX gene activation is likely to extend beyond the subgroup of patients with CCAs, as MLL and HOXA9 were also significantly upregulated in unselected MDS patient samples, including those with normal karyotypes.[37]

HOX Genes: Multiple Roles in Development, Hematopoiesis and Leukemogenesis

Homeobox genes were first recognized through the analysis of homeotic mutations of Drosophila, which alter the identity of various body segments. Homologous genes have been found in virtually every species, from yeast to humans. Class I homeobox genes are designated as *HOX* genes in humans (mouse:*hox* genes). HOX genes control morphogenesis in early stages of embryonic development. The specific shape of discrete segments (pattern formation) is decisively regulated by these genes. Beside their role as differentiation factors in embryonic development, the control of hematopoiesis by *HOX* genes is well established. Since the perturbation of hematopoietic stem cell development is a hallmark of leukemia, it is not surprising that the aberrant expression of *HOX* genes contributes decisively to leukemia pathogenesis.[41]

Early experimental evidence suggesting the oncogenic potential of the *HOX* gene family came from studies showing that the overexpression of Hoxb8 and IL-3 in murine bone marrow cells can induce aggressive, transplantable leukemia. A similar approach showed that Hoxa9 and Hoxa10 are able to induce AML in mice. Retroviral insertional mutagenesis has likewise implicated the Hox genes in leukemia induction. For example, Hoxa7 and Hoxa9 were activated in the context of retrovirally induced AML in BXH-2 mice. The importance of Hoxa7, Hoxa9 and the cofactor Meis1 in the BXH-2 mouse leukemia model was impressively underscored by a study based on large scale cloning of proviral integration sites.

Further compelling evidence of the oncogenic potential of HOX genes comes from their direct or indirect involvement in leukemia-associated translocations, such as the translocation t(7;11)(p15;p15), which generates the fusion protein NUP98-HOXA9 in AML patients. Additional translocations involving HOX loci have been identified over the past years.[42,43] As pointed out above, MLL translocations (6%-7% of all acute leukemia) and most likely also MLL amplifications lead likewise to a dysregulation of HOX gene expression. Very recently CDX4 was shown to regulate expression of HOXA7 and HOXA9.[44] Thus, this homeobox transcription factor and it's relative CDX2 are attractive candidates for unidentified upstream regulators of HOX gene expression in leukemia. Taken together, published reports leave little doubt that specific *HOX* genes, particularly *HOXA7*, *HOXA9* and *HOXA10*, are involved in the pathogenesis of AML and MDS, but virtually nothing is known about the downstream pathways through which these genes exert their oncogenic potential.

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