Comment on Will et al, page 2076

MDS: roadblock to differentiation

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In this issue of Blood, Will et al identify aberrant expansion of distinct stem and progenitors in myelodysplastic syndromes (MDS) and demonstrate that MDS stem cells are functionally abnormal and harbor genetic and epigenetic alterations.1

MDS is thought to be a disorder of hematopoietic stem cells (HSCs).2 Chromosomal abnormalities, mutations, and epigenetic changes have been reported in MDS progenitors.3,4 However, the earliest stage at which these molecular and pathogenic events occur and the functional consequences of these events has not been clearly established. In the present study, Will and colleagues functionally and molecularly characterize highly purified primitive stem cells and progenitors using technically challenging techniques in a variety of MDS subtypes. They show that the primitive long-term stem cell (LT-HSC) pool is significantly increased in high-risk MDS subtypes compared with healthy controls (see figure). Further analysis of committed progenitor populations shows that lower-risk MDS patients have skewed expansion of common myeloid progenitors (CMPs). In contrast, analysis of high-risk patients reveals expansion of the granulocyte-monocyte progenitors (GMPs) with a relative decrease in the megakaryocyte-erythrocyte progenitor (MEP) population. Notably, this concept of differential arrest is not unique to MDS; GMP expansion has recently been reported in acute myeloid leukemia (AML)3 and block in myeloid differentiation is a well-characterized feature of the blastic phase of chronic myeloid leukemia (CML-BP).6

Will and colleagues further investigate the functional and molecular signatures of primitive MDS HSCs. Karyotypic abnormalities are identified in the majority of immature HSCs from patients representing the various MDS subtypes. Importantly, the authors find that these cytogenetic abnormalities persist even in the expanded CMP and GMP populations. Further, they show MDS-HSCs are functionally deficient in their clonogenic potential and give rise to dysplastic colonies. The authors perform additional genome-wide methylomic and transcriptomic analysis of these rare stem cells and demonstrate that primitive MDS-HSCs are not only karyotypically and functionally abnormal but also exhibit widespread alteration in DNA methylation and gene expression. Although epigenetic alterations including hypermethylation have earlier been investigated in CD34+ cells7 and bone marrow cells8 from MDS patients, Will and colleagues are the first to characterize such patterns in primitive stem cells. In addition to identifying a subset of genes that are hypermethylated, they also describe a novel signature of hypomethylated genes in these cells, implicating a potential previously unrecognized role for altered hypomethylation in MDS pathogenesis. To validate these findings functionally the authors perform additional studies for STAT3, which they find to be significantly hypomethylated and overexpressed in HSCs from all tested subsets of MDS. They demonstrate that MDS HSCs are sensitive to pharmacologic inhibition of STAT3 in clonogenic assays, showing reduced colony formation compared with normal HSC controls. Taken together, these findings describe a list of candidate genes (including STAT3) that are dysregulated in primitive MDS stem cells that may serve as a target for stem cell–directed therapies for the disease.

Clinically, the most relevant finding of this study is that this cytogenetically abnormal stem cell pool persisted in a patient with complete morphologic remission after epigenetic therapy with azacytidine and vorinostat. Furthermore, through careful examination of serial samples, the authors demonstrate that morphologic relapse in this patient was preceded by expansion of the HSC compartment. This is consistent with another recent report that found persistence of rare and malignant HSCs in MDS with the 5q− abnormality in


Comment on Gallagher et al, page 2098

Mutation associations in RA-defiant APL

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One-quarter of acute promyelocytic leukemia (APL) patients develop resistance to all-trans retinoic acid (ATRA)/chemotherapy (CT). In this issue of Blood, Gallagher et al report the associations of PML-RARα mutations, FLT3 mutations, and additional chromosome abnormalities (ACAs) in relapsed APL.

Intrinsic or acquired resistance to anticancer drugs can arise from a variety of factors including the development of mutations in drug targets and additional genetic abnormalities. In APL, which is driven by the (t(15;17)-generating PML-RARα, ATRA in combination with CT achieves a complete remission rate of 90% and a 5-year disease-free survival of 74%. However, ATRA resistance has been reported for 2 decades and was shown to be associated with increased catabolism and decreased delivery to cell nucleus of ATRA, as well as mutations in the ligand-binding domain (LBD) of the RARα portion of the fusion protein. In this study, Gallagher et al further dissect the potential association of PML-RARα LBD mutations (PRα/LBD+), FLT3 mutations, and ACAs in relapsed APL on ATRA/CT (see figure).

The authors show that among 45 relapsed patients from the ATRA/CT treatment group, 18 cases harbor PRα/LBD+ (40%), 7 of whom (39%) relapsed more than 30 days after last ATRA dose (off ATRA) selection pressure, suggesting a possible active role of PRα/LBD+. Indeed, Gallagher et al observed in 2 cases the selection of a pre-existing mutant subclone by ATRA that lead to relapse on or off ATRA treatment. Unlike PRα/LBD+, the incidence and quantification of FLT3-ITD+ are not increased during relapse without any evidence of influence of ATRA treatment. The fact that all FLT3-ITD+ patients who relapsed off ATRA lacked PRα/LBD+, and most FLT3-ITD+ patients (83%) who relapsed on ATRA had a coincident PRα/LBD+, suggests that ATRA could not eliminate the double-mutant subclone. Exclusive ACAs are identified at diagnosis, and are significantly increased (2-fold) at relapse (29% to 62%). Structural chromosome changes are predominantly newly present at relapse and differ from ACA at diagnosis. Interestingly, despite the heterogeneity of ACAs, they are associated with a phenotype of pWBClow and L-isoform, if relapse occurred off ATRA. FLT3-ITD+ as a mechanism leading to off-ATRA disease progression may be proved by the observation that ACA-PRα/LBD+ is negatively associated with FLT3-ITD+, which presents an opposite phenotype of pWBChigh and S-isoform. However, in on-ATRA relapsed patients, the above associations are not apparent, suggesting a distinct different mechanism of resistance and progression. In prognostic analysis, only the presence of ACA at relapse is associated with reduced postrelapse outcome. In the patients with PRα/LBD+...
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