IN THE SPOTLIGHT

Soil and Seed: Coconspirators in Therapy-Induced Myeloid Neoplasms

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Summay: In this issue of *Blood Cancer Discovery*, Stoddart and colleagues describe cooperative effects of exposing both the bone marrow microenvironment of recipient mice and donor hematopoietic stem and progenitor cells (HSPC) to an alkylating agent in a genetically accurate model of therapy-induced myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) characterized by chromosome 5q deletions. The authors further implicate senescence of alkylator-treated mesenchymal stem cells (MSC) as contributing to the microenvironmental damage and subsequent therapy-induced myeloid neoplasms (tMN). Loss of *Trp53* function and somatic mutations in other DNA damage response (DDR) genes were associated with overt AML in this model. Together, these studies shed new light on the complex pathogenesis of tMN and establish a robust model for biologic and preclinical investigation.

See related article by Stoddart et al., p. 32 (2).

Therapy-induced malignant neoplasms (tMN) are hematologic malignancies caused by therapeutic exposure to genotoxins used to treat a previous histologically distinct cancer and certain other diseases (reviewed in ref. 1). They are among the most lethal blood cancers, with a 5-year survival of 10% to 20%. While hematopoietic stem cell transplantation is curative in some patients, therapy-induced myeloid leukemia (tML) is frequently associated with advanced age and substantial comorbidities that preclude this therapeutic option. Since the initial reports by Rowley and colleagues in the 1970's of a specific pattern of recurring cytogenetic alterations characterized by loss of chromosomes 5 and/or 7, much has been learned about the epidemiology, genetics, and biology of tML. Specifically, tML can be broadly subclassified into distinct types that arise following exposure to either topoisomerase inhibitors such as etoposide and daunomycin (~30% of cases) or treatment with radiation and/or alkylating agents (the remaining 70%). Patients who develop tML following topoisomerase therapy typically present with acute myeloid leukemia (AML) after relatively short latency (generally around 2 years after the last exposure). Consistent with the molecular mechanism of topoisomerase-induced DNA damage, these AMLs are characterized by chromosomal translocations involving genes such as *KMT2A* (*MLL*), *RUNX1* (*AML1*), and *PML-RARA* that are also altered frequently in *de novo* AML. Cases of tMN that arise after exposure to radiation and/or alkylating agents typically show a longer interval between genotoxin treatment and clinical onset. Patients frequently present with cytopenias due to myelodysplastic syndrome (MDS) and exhibit an aggressive clinical course, including rapid transformation to AML. Monosomy 5 and 7 and large deletions of the long arms of these chromosomes, del(5q) and del(7q), are cytogenetic hallmarks of this type of tMN. Although chromosome 5 and 7 abnormalities coexist in a significant proportion of tMNs with complex karyotypic abnormalities, McNerney and colleagues (1) have distinguished tMNs with del(5q) or monosomy 7/del(7q) from each other based on the presence of underlying risk factors and associated somatic mutations. These myeloid malignancies also share key biologic and genetic features with specific subsets of *de novo* MDS and AML that are predominantly diagnosed in older individuals and are also largely refractory to therapy. An article by Stoddart and colleagues in this issue of *Blood Cancer Discovery* (2) that demonstrates cooperative effects of alkylator exposure on the bone marrow microenvironment and hematopoietic stem and progenitor cells (HSPCs) in a mouse model of del(5q) MDS and AML advances our current understanding of del(5q) diseases and, more broadly, provides new insights into the key role of non-cell–autonomous mechanisms in the pathogenesis of MDS and other myeloid malignancies.

Whereas comprehensive genome-wide sequencing and transcriptome analyses have informed functional studies that elucidated many key "driver" genes, pathways, and molecular mechanisms in hematologic cancers, the *de novo* and alkylator-induced myeloid malignancies characterized by large 5q deletions and monosomy 7/del(7q) remain, in important respects, stubborn outliers. This is due, in large part, to hundreds of 5q or 7q of genes that are deleted in most patients and the absence of frequent "second hit" mutations in any single gene from these large chromosomal intervals. Thus, del(5q) and del(7q) are best viewed as contiguous gene syndromes where haploinsufficiency at multiple individual loci contributes to the overall disease phenotype in the

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context of other mutations and, as seems increasingly likely, stress in the bone marrow microenvironment (1). Moreover, next-generation sequencing unexpectedly failed to support the straightforward model that genotoxins directly mutate key initiating genes in a susceptible HSPC, which then achieves a clonal growth advantage, acquires additional cooperating mutations, and causes leukemia (3). Indeed, the overall mutational burdens are similar in tMN and *de novo* myeloid malignancies, and extensive data now support the alternative idea that genotoxin treatment favors the survival and subsequent outgrowth of clones with preexisting mutations in *TP53* and other DNA damage response (DDR) genes (3, 4). Recent observations in individuals with clonal hematopoiesis of indeterminate significance (CHIP), which is characterized by age-dependent expansion of a clone harboring mutations in known MDS and AML genes such as *ASXL1, TET2, DNMT3A*, or *TP53* in individuals without hematologic abnormalities, provides further support for this mechanism. Specifically, retrospective studies revealed CHIP at the time of the initial cancer diagnosis in many patients who went on to develop tMN (reviewed in ref. 5). In this context, the presence of CHIP may be a marker of a compromised HSPC compartment that is susceptible to genotoxic damage, may indicate the existence of a clone that will acquire a profound fitness advantage in the context of alkylator exposure, or both. Given this complexity, it is perhaps not surprising that much remains to be learned with respect to how chromosome 5 and 7 deletions contribute to leukemogenesis broadly and in tMN.

The new study by Stoddart and colleagues reports a comprehensive series of experiments in a murine adoptive transfer model of tMN characterized by haploinsufficiency for *Apc* and *Egr1* and by shRNA-mediated knockdown of *Trp53* in donor HSPCs. Haploinsufficient loss of *APC* and *EGR1* occurs in the vast majority of tMNs with a del(5q), and 75% of patient samples also harbor *TP53* deletions or mutations. The authors first isolated HSPCs from compound *Egr1*$^{+/-}$; *Apc*$^{+/-}$ (*EA*)–mutant mice, infected them with a validated *Trp53* shRNA construct that also encodes a *GFP* marker gene, and injected these cells into lethally irradiated wild-type (WT) recipients. Remarkably, exposing both donor and recipient mice to N-ethyl-N-nitrosurea (ENU) 2 to 3 weeks prior to adoptive transfer greatly accelerated the development of aggressive hematologic malignancies. The authors went on to show that this was dependent on exposing *both* donors and recipients to ENU and, further, that ENU treatment strongly promoted the outgrowth of GFP-positive cells that had been transduced with the *Trp53* shRNA. This observation that alkylator exposure selects for the outgrowth of *TP53*-mutant cells is consistent with serial sequencing of some human tMNs and with *in vivo* competitive growth assays in mice (3, 4). In addition to showing reduced survival, donor/recipient pairs that were exposed to ENU developed MDS and AML with high penetrance. These AMLs were fully transplantable into lethally irradiated secondary recipients in the absence of ENU. Together, these data support the idea that alkylator-induced alterations create a permissive bone marrow environment for the survival and outgrowth of cells that ultimately give rise to tMNs. Postinitiation events that drive progression to AML reduce or eliminate dependence on these key microenvironmental signals.

Focusing on potential cellular targets of ENU in the bone marrow microenvironment, the authors showed that ENU exposure both reduced the proliferation of cultured mesenchymal stem cells (MSC) and promoted their senescence in a dose-dependent manner. They extended these studies by demonstrating that the MSCs isolated from mice that were exposed to ENU exhibited reduced colony-forming unit fibroblast growth as well as transcriptional changes such as activation of p53-regulated genes and of senescence-associated secretory expression phenotype. Consistent with a functional role of a stressed microenvironment in clonal selection, ENU treatment of recipient mice also enhanced the competitive fitness of *Ergr*$^{+/-}$ HSPCs.

In a final series of studies, the authors addressed the role of *Tp53* knockdown in the context of ENU treatment. They found that *EA* HSPCs from both untreated and ENU-treated donors efficiently induced MDS in ENU-treated WT recipients in the absence of *Trp53* knockdown, with a median latency of >1 year. In contrast, injecting HSPCs from these ENU-treated mice into untreated WT recipients only rarely resulted in hematologic disease. These studies reinforce the central role of alkylating agent–mediated changes in the microenvironment of recipient mice in disease initiation. Transferring *EA* cells that were also infected with the *Trp53* shRNA markedly accelerated disease onset in the context of ENU exposure (median onset 200 days) and induced progression to AML or T-cell malignancies in most of the recipients. In comparison with MDS bone marrow cells isolated from recipient mice that received *EA* cells without *Trp53* knockdown, these AMLs strongly upregulated gene sets associated with early stem progenitor cells, *MYC* pathway activation, and WNT/β-catenin signaling. Importantly, the same gene sets are enriched in del(5q) tMN patient samples. Finally, while comparative next-generation sequencing revealed a subtle and statistically insignificant difference in the overall mutation burdens in *EA* MDS and *EA* + *Trp53* knockdown AML samples (8 vs. 12.5 per case), the AMLs were enriched for mutations in genes broadly associated with DDR and signal transduction. Although *TP53* is rarely comutated with other DDR genes in human 5q leukemias, this discrepancy may be due to the use of a knockdown approach, as common p53-mutant proteins have dominant negative activity in myeloid leukemia cells (6).

There is emerging evidence that alterations in the bone marrow microenvironment can, in at least some cases, contribute to the development of hematopoietic malignancies. For example, germline mutations of *SBDS*, present in most patients with Shwachman Diamond syndrome, result in impaired stromal cell function that contributes to the high rate of MDS/AML in this syndrome (7). There also is evidence that malignant hematopoietic cells can induce changes in bone marrow stromal cells that confer a competitive advantage to the malignant HSPCs and/or render them less sensitive to chemotherapy (reviewed in ref. 8). Stoddart and colleagues provide evidence that environmental stressors, in this case, alkylator therapy, also may induce alterations in the bone marrow stromal cells that contribute to the development of myeloid malignancy. Specifically, in their genetically accurate mouse model of del(5q) tMN, alkylator-induced changes in bone marrow stromal cells appears to be a key initiating event, possibility by inducing senescence of MSCs, which, in turn, secrete proteins that support the

development of tMN through direct or indirect mechanisms. This abnormal microenvironment is dispensable after progression to AML, and these later events in tMN pathogenesis are driven, in part, by loss of p53 function and by mutations in other DDR genes. The transplantable AMLs generated here are a novel resource for testing candidate therapies for tMNs with (del)5q.

This work also raises new questions around how MSCs (and perhaps other cells in the microenvironment) create a milieu that is conducive to the development of tMN. Do the stromal alterations induced by alkylator therapy produce a hostile environment that suppresses normal HSCs, or do these changes selectively drive the expansion of mutant HPSCs? Are the stromal alterations induced in mice with ENU also induced by other types of chemotherapy? Does the duration of and/or the intensity of chemotherapy determine the degree and/or type of stromal alterations? How long do the stromal alterations persist after chemo/radiotherapy? Of note, in this study, the ENU-induced stromal changes appear to persist for at least months in mice. A key challenge in extending this work to human tMNs will be to dissect direct effects of genotoxic stress on HPSCs from the indirect effects of an abnormal microenvironment on disease initiation and clonal outgrowth. It is also unclear whether chromosome 5q deletions, and haploinsifficiency for *EGR1, APC*, and other embedded genes, comprise early or late events in this process. Finally, are the findings of this study generalizable beyond this model system and/or cases of tMN associated with del(5q) and impaired p53 function?

With respect to this last question, we conclude with recent insights into the pathogenesis of myeloid malignancies characterized by monosomy 7/del(7q), which is the other hallmark genetic aberration in alkylator-induced tMN (reviewed in ref. 9). There are clear differences in the respective genetic landscapes of tMNs with monosomy 7/del(7q) or del(5q), with *TP53* mutations rare in tMNs with monosomy 7/del(7q) and Ras pathway mutations far more common. In addition, monosomy 7/del(7q) is associated with a number of inherited and environmental predispositions to myeloid malignancies. Recent studies demonstrating causative germline mutations in the *SAMD9* and *SAMD9L* genes, which are located on chromosome band 7q11, as a major cause of familial MDS and AML unexpectedly showed that they encode gain-of-function proteins that suppress cell growth in response to IFNs and other proinflammatory stimuli. Accordingly, these alleles are unexpectedly lost in the monosomy 7 cells of patients with hematologic disease due to "adaptation by aneuploidy" (9). Additional observations about these genes include (i) the parents of children who develop MDS or AML with monosomy 7 and harbor the same germline *SAMD9/9L* mutation typically have normal blood counts in association with somatic uniparental disomy (UPD) or revertant mutations in the hematopoietic compartment (10); (ii) similarly, monosomy and hematologic abnormalities resolve spontaneously in many children with outgrowth of a clone that exhibits somatic UPD or a revertant mutation; (iii) the bone marrows of children who progress to MDS and AML acquire secondary mutations in genes that are also associated with monosomy 7 in *de novo* myeloid malignancies, including *SETBP1* and *RUNX1;* and (iv) *Samd9* functions as a haploinsufficient TSG in mice (there is no mouse *Samd9* gene; ref. 11). Together, these observations support the idea that the SAMD9/9L path way functions to protect the HSPC compartment from microenvironmental stress in a manner that is perhaps functionally analogous to the role of p53 as "guardian of the genome." Mutant *SAMD9/9L* alleles encode proteins that "overshoot" in response to inflammatory stress and dominantly suppress HSPC function. This, in turn, confers a fitness advantage in an HSPC that loses the mutant allele and the respective chromosome 7 homolog. Given these observations, it is tempting to speculate that prolonged inflammatory stress in the bone marrow microenvironment both leads to loss of normal HPSCs and promotes the outgrowth of rare monosomy 7 clones that are haploinsufficient for *SAMD9* and *SAMD9L* in other contexts, including germline *GATA2* mutations, cases of MDS arising after immunosuppressive treatment for aplastic anemia, and in t-MN. In conclusion, while much remains to be learned, the elegant study by Stoddart and colleagues and recent observations in familial MDS characterized by somatic monosomy 7 have converged on the bone marrow microenvironment as fertile "soil" for future investigation into the mechanisms underlying tMN and *de novo* myeloid malignances.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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