



MYELODYSPLASTIC SYNDROMES

The mesenchymal niche in MDS

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Myelodysplastic syndrome (MDS) is characterized by bone marrow failure and a strong propensity for leukemic evolution. Somatic mutations are critical early drivers of the disorder, but the factors enabling the emergence, selection, and subsequent leukemic evolution of these “leukemia-poised” clones remain incompletely understood. Emerging data point at the mesenchymal niche as a critical contributor to disease initiation and evolution.

Disrupted inflammatory signaling from niche cells may facilitate the occurrence of somatic mutations, their selection, and subsequent clonal expansion. This review summarizes the current concepts about “niche-facilitated” bone marrow failure and leukemic evolution, their underlying molecular mechanisms, and clinical implications for future innovative therapeutic targeting of the niche in MDS. (*Blood*. 2019;133(10):1031-1038)

MDS: a disease of genetic events in HSCs, but what is missing?

Myelodysplastic syndrome (MDS) is a clonal disorder of hematopoiesis that is characterized by bone marrow failure (BM) and the propensity for evolution toward acute myeloid leukemia (AML). The disorder is driven by (epi)genetic events in hematopoietic stem and/or progenitor cells (HSPCs).¹ Emerging insights have revealed that acquisition and selection of “poising” mutations in epigenetic regulators, such as *DNMT3A*, *TET2*, *ASXL1*, and splice factors, are critical first steps in the pathogenesis of MDS.² HSPCs in MDS typically acquire genetic lesions that, in concert, drive malignant transformation. It remains incompletely understood how a single hematopoietic stem cell (HSC) that is generally considered to be in a predominantly quiescent state acquires multiple mutations. Also, it is not immediately apparent why some “founding” mutations, in genes like *ASXL1* and splice factors, that do not result in a cell-intrinsic competitive advantage over nonmutated HSPCs persist in the BM and eventually result in clonal outgrowth.³⁻⁶ Finally, it is not immediately clear how relatively minor mutated clones, with consequently considerable remaining residual normal hematopoiesis, result in cytopenia in MDS. In obtaining insight into these open questions, Darwinian evolution principles predict that a cell’s direct environment, or HSPC niche, may play critical roles in the functional competence of HSPCs and the emergence and selection of genetic clones within a population.

A role for the niche? Circumstantial support for a role for the niche in MDS pathogenesis

MDS has long been considered a hematopoietic cell-autonomous disorder in which disease initiation and progression are exclusively driven by hematopoietic cell-intrinsic genetic events. However, several earlier observations have challenged this reductionist view

and suggested a contribution of the BM microenvironment to disease pathogenesis. Bone abnormalities suggesting “adynamic” bone, characterized by reduced osteoblast numbers, decreased mineral apposition rates, and osteoporosis, have been noted in MDS patients in comparison with age-matched controls.^{7,8} Early histologic findings pointed to the disruption of the BM architecture as a common finding in MDS (see Raaijmakers⁹ for additional details). BM-derived stromal cells (BMDSCs), a readily available source of stromal cells from the hematopoietic environment, have subsequently been examined as a component of this disrupted architecture. These plastic-adherent ex vivo-expanded cells expressing defined stromal markers display transcriptional abnormalities, altered differentiation characteristics, and a reduced ability to support HSPCs in MDS (see Raaijmakers⁹ and Li and Calvi¹⁰ for additional details), suggesting a potential role in BM failure in MDS. Critical contributions of the BM microenvironment have further been suggested by the inability to propagate human MDS cells in a cell-autonomous manner.¹¹ Finally, the intriguing phenomenon of donor cell-derived hematopoietic neoplasm, defined as oncogenic transformation of apparently normal transplanted donor cells in the allogeneic patient environment, but not in the donor,¹² suggests a potential contribution of ancillary cells to disease initiation of MDS. Albeit infrequent, these cases seem to defy the dogma that events initiating MDS and its defining characteristics are completely hematopoietic cell autonomous. Some investigators have suggested that the curative potential of allogeneic HSPC transplantation argues against pivotal contributions of the BM niche to disease initiation and progression. Cure, however, remains infrequent; in these patients, a necessary (but not sufficient) contribution of the BM microenvironment to disease pathogenesis, in cooperation with genetically aberrant HSPCs, cannot be excluded. Collectively, these observations and considerations challenge the view that ineffective hematopoiesis and leukemic transformation are exclusively driven by hematopoietic cell-autonomous events in human MDS and formed the rationale for experimental approaches to

better define a potentially causative or permissive role of the microenvironment in human MDS in the last decade.

Experimental definition of niche contributions to MDS

The identification of functional components of the HSC niche at cellular resolution in mice^{13,14} has enabled experiments addressing their contributions to hematopoietic disease. Experimental support for a potential pivotal contribution of the BM niche to the pathogenesis of MDS was first provided in a study showing that dysfunction of a well-defined stromal subset of bone progenitor cells can initiate myelodysplasia and leukemia predisposition.¹⁵ Targeted deletion of the microRNA-processing endonuclease *Dicer1* from osterix-expressing osteoprogenitor cells, but not mature osteoblasts, recapitulated key features of human MDS. This included the propensity to develop, at low frequency, AML harboring distinct cytogenetic abnormalities. *Dicer1*-deleted osteoprogenitor cells expressed reduced levels of *Sbds*, the gene mutated in Shwachman-Diamond syndrome (SDS), a human congenital BM failure and leukemia-predisposition disorder. Deletion of *Sbds* from osteoprogenitor cells phenocopied myelodysplasia in mice. The data demonstrated the central role that individual cellular elements of “stroma” can play in tissue homeostasis and revealed that primary dysfunction of such cells can initiate secondary neoplastic disease in the hematopoietic system. The work postulated a “niche-based” model of oncogenesis whereby an altered microenvironment cell can initiate a multistep process toward malignancy in hematopoietic cells.

The work furthered earlier studies in mice pointing at a potential driving impact of external signaling in the initiation of hematopoietic disease. Deletion of specific genes from the microenvironment, including *RARγ* and the inhibitor of NF-κB (*IκBa*), resulted in myeloproliferative neoplasms (MPNs)¹⁶ and a chronic myelomonocytic leukemia-like disease¹⁷ via *Notch 1* activation in hematopoietic cells, respectively

More recently, murine studies have more specifically implicated mesenchymal cells in the pathogenesis of human MDS and related disorders. An activating mutation of β-catenin in mesenchymal cells resulted in the development of myelodysplastic alterations and AML with common genetic aberrations.¹⁸ Activated β-catenin stimulates expression of *jagged1* in mesenchymal cells, with subsequent activation of Notch signaling in HSPCs driving leukemic evolution. Human disease relevance was suggested by the increased β-catenin signaling and nuclear accumulation in osteolineage cells in a subset of MDS patients. Genetic or pharmacological inhibition of Notch signaling ameliorates AML and demonstrates the pathogenic role of the Notch pathway.

Mutations in *Ptpn11* in mesenchymal precursor cells (MPCs), in the context of the congenital Noonan syndrome, promoted a juvenile myelomonocytic leukemia (JMML)-like disease and caused donor cell-derived MPN following stem cell transplantation.¹⁹ Similarly, deficiency of *Sipa1* in the microenvironment is sufficient to drive an MDS/MPN-like phenotype in mice, with resemblance to the *Dicer1* model, including reduction of B-cell progenitors, impaired osteogenic potential of MPCs, and

the occurrence of myelodysplastic features in hematopoietic cells.²⁰

Relevance of mesenchymal contributions to human MDS was further confirmed in experiments showing that patient-derived BMDSCs display a disturbed differentiation program and are essential for the propagation of MDS-initiating Lin⁻CD34⁺CD38⁻ stem cells in xenograft models.²¹ Coinjection of CD34⁺ hematopoietic cells obtained from lower-risk (LR) MDS patients, together with their corresponding in vitro-expanded mesenchymal stromal cells, into the marrow cavity of NOD/LtSz-*scid-IL2rg*^{-/-} mice significantly increases reconstitution with human MDS cells in comparison with transplantation with CD34⁺ alone or coinjection with BMDSCs from age-matched normal BM.

Finally, in vivo data in a transgenic murine model of *NUP98-HOXD13* (*NHD13*) driven MDS have supported the relevance of the BM microenvironment in disease pathogenesis, because transplantation of *NHD13* hematopoietic cells into wild-type recipients compared with *NHD13* recipients resulted in a lower rate of leukemic progression and death, as well as improved hematopoiesis.²²

Taken together, the data provide strong support for the conceptual view of “niche-driven malignant transformation,” in which primary alterations in niche cells drive the dysfunction and malignant transformation of supported, but distinct, HSPCs. Vice versa, primary (epi)genetic alterations in hematopoietic cells have the ability to alter mesenchymal niche components, such that niche cells facilitate disease propagation, supporting a concept of “niche-facilitated malignant transformation.”²³⁻²⁷ Importantly, the molecular mechanisms driving these biologic concepts may be overlapping, as discussed in the next section.

What are the molecular mechanisms driving niche contributions to MDS pathobiology?

The mouse experiments providing proof of principle of niche contributions to MDS pathogenesis have generated important insights into the underlying molecular mechanisms. Here, we will discuss the pathways that have emerged as key drivers of the MDS phenotype, supported by in vivo data and complemented by observations in human MDS.

Activation of inflammatory programs in MPCs: insights from congenital leukemia predisposition syndromes

Congenital monogenic BM failure syndromes with leukemia predisposition have provided unprecedented models to interrogate the contribution of the niche to MDS and leukemic evolution.

TP53-s100A8/A9-Toll-like receptor 4 signaling SDS is caused by constitutive biallelic loss-of-function mutations in the ribosome biogenesis gene *SBDS*. Targeted deletion of *Sbds*, specifically in MPCs, faithfully recapitulates human disease.²⁸ The *Sbds*-deficient environment induced mitochondrial dysfunction, oxidative stress (induction of reactive oxygen species [ROS]), and DNA damage in HSPCs. This was achieved via secretion of an array of inflammatory ligands, including the alarmins S100A8 and S100A9, downstream of *Tp53* activation, ultimately resulting

in *Tp53*-activation-associated apoptosis in HSCs, in part via TLR4 signaling. Transcriptional activation of this signaling axis in the mesenchymal niche was found in a subset of LR MDS patients, and it predicted leukemic evolution and progression-free survival. The data support the idea that inflammatory alterations in the mesenchymal niche attenuate the functional integrity of HSCs and may facilitate genetic alterations and subsequent transformation.

NF- κ B-chemokine (CCL3)-IL-1 β signaling The notion that inflammatory alterations in mesenchymal niche cells may drive leukemic evolution was further supported by a study demonstrating a pivotal contribution of the mesenchymal niche to the genesis of JMML in Noonan syndrome.¹⁹ Mechanistically, inflammatory alterations, including *Ccl3* upregulation in mesenchymal cells, recruited cells of the myeloid lineage to putative HSC niches. Subsequently, HSCs are “hyperactivated” by interleukin-1 β (IL-1 β) and possibly other proinflammatory cytokines. It appeared that persistently high levels of proinflammatory cytokines produced by the recruited monocytes displaced HSCs from MPC niches that are essential for maintaining HSC dormancy, resulting in their activation and, ultimately, donor cell-derived MPN in a transplantation-based mouse model. Administration of *Ccl3* receptor antagonists effectively reverses the development of JMML-like disease induced by the *Ptpn11*-mutated BM microenvironment.

Overexpression of *Ccl3* and other inflammatory factors in mesenchymal niche cells has been reported in a variety of mouse models of myeloid malignancies,²²⁻²⁴ including the SDS niche model,²⁸ as well as mesenchymal cells of MDS patients.^{29,30} Highly purified CD271⁺ primary mesenchymal elements from LR MDS patients display global activation of inflammatory programs with a remarkable abundance of signatures related to NF- κ B, EGF, tumor growth factor- β , and tumor necrosis factor signaling.²⁹ Inflammatory alterations include upregulation of *CCL3*, *TGF β* , *IL6*, and *IL8* and a wide variety of factors previously demonstrated to be negative regulators of hematopoiesis, in particular erythropoiesis and B lymphopoiesis, differentiation programs typically affected in LR MDS. It is conceivable that the activin receptor type II ligand traps, such as sotatercept, exert part of their clinical effect through modulation of such inhibitory stromal factors.³¹ Mesenchymal NF- κ B activation is a driver of the transcriptional upregulation of these inflammatory programs in LR MDS patients and results in *ex vivo* attenuation of HSPC numbers and function.³² These findings in human MDS complement murine studies implicating NF- κ B activation in ancillary cells as a driver of “MDS/MPN-like” disease^{17,33} and may be seen in the context of NF- κ B-driven chronic tissue inflammation as a driver of cancer initiation and progression via secretion of cytokines and soluble factors in other solid cancer models.^{34,35}

Together, the data indicate that inflammation, specifically driven by MPCs in the hematopoietic niche directly or indirectly, leads to attenuation of HSC homeostasis and facilitates neoplastic transformation of the BM.

Inflammation in the BM in MDS: location may matter The HSPC niche is an anatomically defined regulatory environment that governs the behavior of HSPCs. It is important to note that, although inflammatory alterations in the BM in MDS likely involve many cell types (including innate and adaptive immune cells), the proximity of such an inflammatory cell to (leukemia-

initiating) HSPCs may be pivotal in its contribution to malignant transformation. Inflammatory ligands, coming from niche cells and other hematopoietic subsets present in the BM, may exert their biological effects specifically in the vicinity of the producing cell. For example, the local accumulation of S100A8/A9 in the environment is very high (up to 100 mg/mL and ~50- to 100-fold higher than systemic concentrations), likely caused by attachment to extracellular matrices, such as proteoglycans.³⁶ This implies that the exposure of HSPCs to S100A8/A9 is projected to relate strongly to their anatomical proximity to a producing cell. CD271⁺ mesenchymal cells are directly adjacent to CD34⁺ HSPCs in human BM.³⁷ This notion of “spatial relevance” of inflammatory alterations in the BM may also be congruent with recent observations that aberrant overexpression of S100A8/A9 in hematopoietic/erythroid cells within the erythroid island niche in a model of human del(5q) MDS leads to a predominant erythroid anemic phenotype.^{38,39} The combined data suggest that similar inflammatory axes may determine distinct aspects of the MDS phenotype related to distinct niches.

Activation of Wnt signaling in mesenchymal cells

The finding that an activating mutation in β -catenin in mouse osteolineage cells can be the initiating event in MDS/AML¹⁸ should be interpreted in the context of a number of other studies pointing at a potential role for activation of β -catenin signaling in the mesenchymal environment in the pathogenesis of MDS.

Mice with haploinsufficiency for *Apc* develop an MDS-like phenotype that is characterized by macrocytic anemia and monocytosis.⁴⁰⁻⁴² Again, MDS is driven by the microenvironment in these mice, as demonstrated by the finding that transplantation of normal hematopoietic cells into lethally irradiated *Apc*^{del/+} recipients resulted in a fatal macrocytic anemia. Transplantation of *Tp53*^{+/-} donor cells into *Apc*^{del/+} mice resulted in the development of an immature lymphoid leukemia, suggesting that the microenvironment is conducive to developing neoplastic disease.⁴³ Loss of 1 copy of *Ctnnb1* was sufficient to prevent or delay the development of MDS in these mice, providing more formal genetic proof that altered canonical Wnt signaling in the microenvironment was responsible for the disease.⁴² Pyriminyl, a US Food and Drug Administration-approved drug, dampened Wnt signaling in mesenchymal niche cells isolated from this mouse model and patients with del(5q) MDS and delayed/inhibited disease in *Apc*^{del/+} mice, even when it was administered after the presentation of anemia.

Activated WNT signaling in mesenchymal cells in MDS may result from epigenetic alterations induced by aberrant hematopoietic cells, as suggested by a study interrogating the DNA methylome of BMDSCs from MDS patients.⁴⁴ This revealed widespread aberrant cytosine hypermethylation, including the WNT pathway antagonist *FRZB*, which was underexpressed in MDS-derived mesenchymal cells. Hypermethylation of cytosine guanine dinucleotides in the *FRZB* promoter could be induced in mesenchymal cells by coculture with leukemic cells (KG1A) and led to activation of WNT in HSPCs. A WNT/ β -catenin-activated signature was also found in CD34⁺ cells from advanced cases of MDS, associated with adverse prognosis. Constitutive activation of β -catenin in hematopoietic cells yielded lethal myeloid disease in a *NUP98-HOXD13* mouse model of MDS, confirming its role in disease progression. Interestingly, the hypomethylating

agent 5-azacitidine may exert part of its clinical effect through modulation of WNT signaling in mesenchymal cells.⁴⁴

Taken together, the data point to activation of WNT signaling in mesenchymal cells as a driver of BM failure and malignant transformation in MDS. Downstream signaling pathways remain largely undetermined, but they may include heterotypic NOTCH and WNT activation in HSPCs, targets for pharmacologic modulation, at least in the experimental setting of mouse modeling.

Loss of mesenchymal support for normal hematopoiesis

Neoplastic HSPCs may damage the HSC niche, undermining the support for residual normal HSCs and, through this mechanism, promote a competitive advantage. Limited data suggest that the frequency of mesenchymal cells is not reduced in MDS.^{29,45} However, in many studies, BMDSCs from MDS patients display reduced proliferative potential, cellular senescence, and reduced hematopoietic support.^{29,46-48} Also, reduced expression of HSC maintenance factors, such as *CXCL12*, has been reported in mesenchymal cells in mouse models^{19,22} and human disease.²⁹ Although cellular senescence in mesenchymal cells awaits confirmation in nonexpanded mesenchymal cells in MDS, the potential relevance is stressed by recent findings in mouse models of myeloid neoplasm. In MPN, sympathetic nerve fibers supporting mesenchymal stem cells are consistently reduced in patients and mice expressing the *JAK2(V617F)* mutation in HSCs.²⁴ MSC reduction is caused by BM neural damage and Schwann cell death triggered by IL-1 β produced by mutant HSCs. AML leads to remodeling of vasculature caused by the production of proinflammatory and antiangiogenic cytokines that gradually degrades endosteal endothelium, mesenchymal cells, and osteoblasts.²⁵ This damage to the endothelial and mesenchymal niches results in reduced support for residual normal hematopoiesis and progression to leukemia. Similarly, in xenograft models of AML, disruption of the adipogenic niche resulted in impaired erythropoiesis and myelopoiesis.²⁶ Finally, it was shown in *Flt3-ITD* mice that *Flt3-ITD*-expressing cells induced a reduction in mesenchymal and endothelial cells with increased inflammation-associated gene expression, resulting in disease progression at the expense of normal hematopoiesis.²⁷

Importantly, these proposed molecular mechanisms underlying niche-facilitated BM failure and leukemic evolution may not be mutually exclusive. WNT/ β -catenin signaling can be upregulated in inflammatory processes.⁴⁹ NF- κ B, through inhibiting glycogen synthase kinase-3 β , activates the canonical WNT pathway.⁴⁹ Paracrine inflammatory signaling converging on the NF- κ B pathway has further been linked to the induction of cellular senescence.⁵⁰⁻⁵² In addition, activation of NF- κ B in mesenchymal cells results in cellular stress and impaired proliferation of mesenchymal cells.³² Similarly, tumor growth factor- β signaling is induced in primary mesenchymal cells from MDS patients and may induce cellular senescence in BMDSCs.⁵³ Cellular senescence, in turn, may develop a chronic prosecretory state, termed the senescence-associated secretory phenotype,⁵⁴ comprising a plethora of inflammatory ligands that play a major stimulatory role in cancer progression.

Collectively, the data suggest that an intricate network of inflammation, NF- κ B, and WNT/ β -catenin activation and cellular

senescence may create a local environment promoting HSC dysfunction and leukemic transformation in MDS (Figure 1).

Niche abnormalities in MDS: chicken or egg?

Although it has become evident that molecular alterations in mesenchymal cells in MDS exist and contribute to disease pathogenesis, it remains unclear whether those alterations are cell intrinsic or induced by aberrant hematopoietic cells. The answer to this question is likely to be context dependent. In the congenital BM failure and leukemia-predisposition syndromes, the primary event in niche cells is the germline genetic abnormality defining the disorder. Mouse models have demonstrated that these genetic alterations in mesenchymal cells are sufficient to drive the hematopoietic phenotypes.^{19,20} In the case of acquired disorders, such as MDS, this “chicken or egg” question is more complicated to answer.

Evidence for genetic alterations in mesenchymal cells in MDS?

Mesenchymal cells in the BM are derived from a common progenitor cell that replenishes stromal elements with potentially differing functions.⁵⁵ A mutation in a mesenchymal stem/progenitor cell may be propagated in its progeny, thus creating a niche field with aberrant biological behavior. This may evoke localized signaling in the niche, ultimately resulting in a local “mutagenic environment” driving malignant transformation or clonal selection of hematopoietic cells. Although conceptually attractive, this series of events would be very difficult to capture in situ in humans if restricted to a very small area of the BM.

Distinct cytogenetic abnormalities in BMDSCs from MDS patients have been reported,⁵⁶⁻⁵⁹ but the significance of these findings is uncertain. It is not always clear whether these alterations exist in situ or have been caused by serial ex vivo passaging of cells. Even if present in the MDS marrow, they may have originated from coevolution in a mutagenic environment rather than precede clonal evolution of hematopoietic cells. Finally, these cytogenetic aberrations have not been linked to aberrant signaling and downstream biological events relevant to the pathogenesis of MDS. Taken together, there seems to be insufficient experimental support for the view that somatic mutations or other genetic events in mesenchymal cells are the primary event in MDS and/or drive disease phenotypes.

However, it is conceivable that primary alterations in the mesenchymal niche, if they exist, are induced by exposure to environmental insults, such as toxic agents, including chemotherapy and irradiation.⁶⁰⁻⁶² Moreover, aging is known to affect mesenchymal cell biology and elicit inflammatory responses from these cells,^{63,64} including alterations implicated in MDS pathology, such as activation of Wnt signaling and *S100A8/A9* overexpression.^{65,66}

Niche alteration as a consequence of genetic events in hematopoietic cells

Although primary (insult or aging-induced) genetic or epigenetic alterations may occur in mesenchymal cells in MDS, it seems more likely that primary genetic alterations in hematopoietic cells induce disruption of niche cells. Comparison of mesenchymal cells

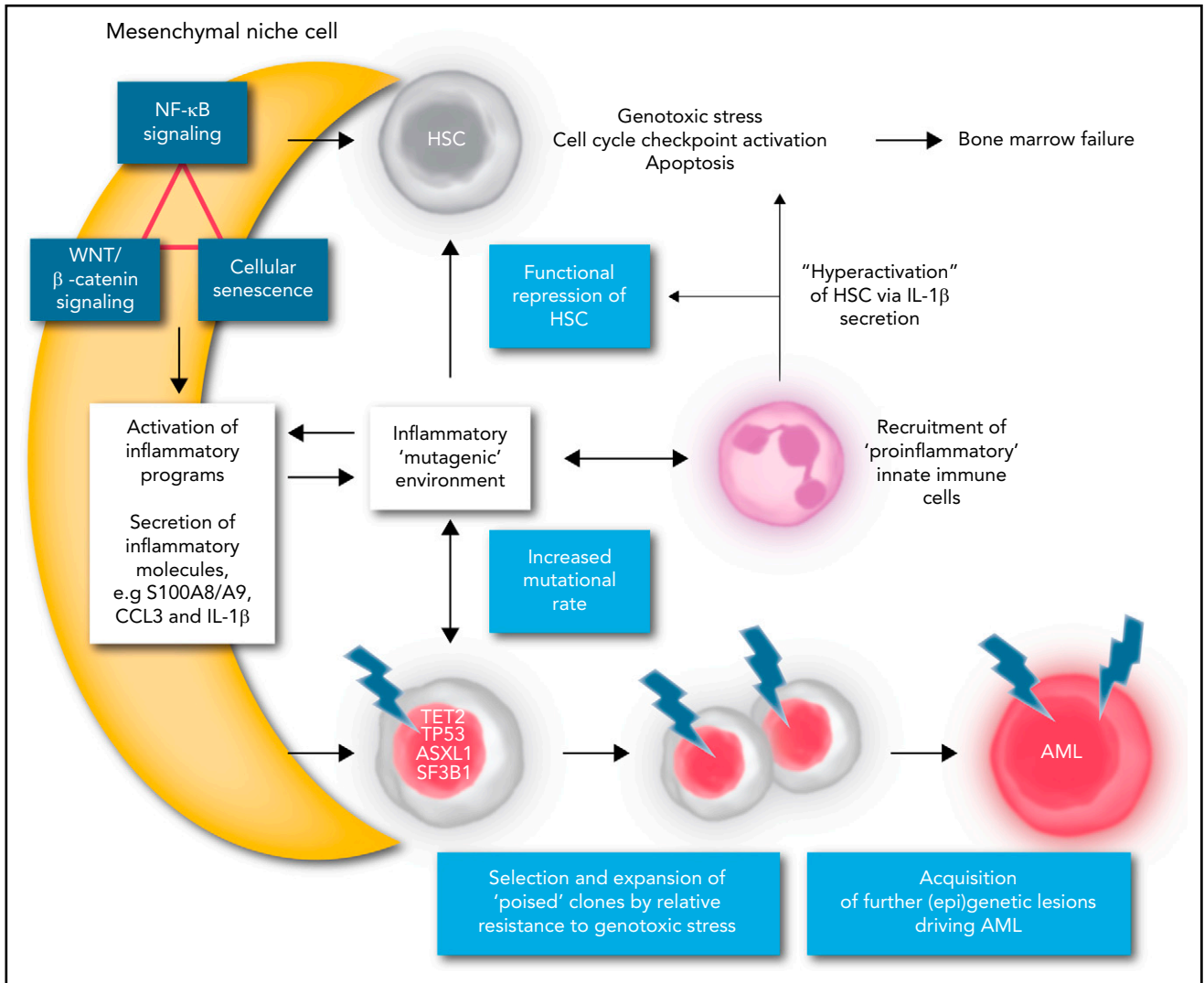


Figure 1. A model of mesenchymal niche-facilitated clonal evolution in MDS. NF- κ B activation, β -catenin/WNT activation, and/or senescence-associated signaling may induce inflammatory alterations in mesenchymal niche cells that result in secretion of inflammatory ligands, such as S100A8/A9, C-C chemokines, and IL-1 β in the HSPC niche. Mesenchymal inflammation may be the result of primary (genetic or epigenetic) alterations in mesenchymal cells or be induced by inflammatory alterations in clonal hematopoietic cells “starting the fire.” The local inflammatory niche induces functional repression of HSCs through direct receptor-mediated genotoxic stress and/or recruitment of proinflammatory innate immune cells, further providing an inflammatory feed-forward loop. The inflammatory stem cell niche may drive the accelerated emergence of mutations in HSPCs through direct genotoxic signaling or the induction of replicative stress. Cells harboring “poising” mutations (ie, mutations that confer resistance to inflammation-induced apoptosis) are selected for in the inflammatory environment by virtue of their relative resistance to inflammation-associated genotoxic stress. The presence of a poising mutation, in an inflammatory environment, accelerates the acquisition of additional genetic events and transformation to frank AML. Thus, inhibition of inflammatory signaling, targeting ligands such as S100A8/A9, C-C chemokines, and IL-1 β signaling or related receptors, is anticipated to alleviate the genotoxic stress on normal HSPCs, relieve the selective pressure on preleukemic clones, and attenuate clonal evolution and leukemic progression in MDS.

directly isolated from BM samples with their *ex vivo*-expanded counterparts (BMDSCs) indicated that many inflammatory programs are tissue context dependent,²⁹ suggesting that interaction with other cells within the MDS BM is required to induce and maintain aberrant molecular programs. This view has been substantiated by coculture experiments in which healthy BMDSCs adopt aberrant MDS MSC-like molecular features when exposed to MDS hematopoietic cells, indicative of an instructive remodeling of the microenvironment.²¹ Those hematopoietic cell-induced changes may include epigenetic alterations,⁴⁴ perhaps explaining why some features of MDS-derived BMDSCs are maintained through serial passaging. Future experiments would have to establish which hematopoietic cell subsets are involved in the cross talk with mesenchymal

cells and whether this signaling is mutation specific. It is conceivable that inflammatory alterations in MDS HSPCs “start the fire” and subsequently induce inflammatory alterations in the mesenchymal niche. Indeed, MDS HSPCs manifest NLRP3 inflammasome activation³⁹ (which may be a direct consequence of an oncogenic mutation, as has been demonstrated for *TET2*²⁷) that directs the generation of IL-1 β , S100A9, and ROS. S100A9-induced signaling, in turn, activates reduced NAD phosphate oxidase and increases levels of ROS that initiate β -catenin activation.³⁹ Moreover, in a recent mouse model of del (5q) MDS, genetic alterations in hematopoietic cells were shown to result in an induction of S100A8/A9 expression in mesenchymal cells.⁶⁸ Thus, one can envision a bidirectional amplifying inflammatory circuitry between mutated MDS cells and their

mesenchymal niche driving and sustaining inflammation in MDS (Figure 1).

Niche-facilitated disease evolution in MDS: induction or selection of mutant clones?

Loss of quiescence, increased proliferative stress, and genotoxic stress or “hyperactivation” induced by alterations in the mesenchymal niche^{19,28} may poise HSPCs for increased DNA damage, promoting malignant transformation. As such, the niche may act as an initiator of oncogenesis. Alternatively, the inflammatory microenvironment in MDS may enable the expansion and, eventually, leukemic transformation, of preexisting genetic clones in the BM. Niche-derived inflammatory signaling may drive the functional repression of HSCs and the subsequent selection and clonal evolution of cells carrying a mutation, conferring resistance to this stress at the cost of “poising” the cell for malignant transformation (Figure 1). In this view, the microenvironment is a “facilitator” that is required, but not sufficient, for malignant transformation.

Clonal selection in the context of microenvironmental alterations has been documented in the context of ageing, toxic insults, and inflammation.⁶⁹⁻⁷¹ Irradiation of mice promotes the selection of particular oncogenic mutations,⁷² including *Tp53*⁷³; this is congruent with the fact that selection for *TP53* mutations occurs during chemotherapeutic treatment and is relevant for the development of therapy-related AML.⁷⁴ Similarly, ageing-associated chronic inflammation might be a driver of clonal selection,⁷⁵ promoting leukemogenesis by providing an environment that is permissive to the expansion of “poised” preleukemic cells carrying founding mutations. For example, B-progenitor fitness reductions and increased selection for oncogene-bearing clones have been shown to be mediated by the inflammatory environment in aged mice.⁷⁶ An inflammatory microenvironment may also induce the selection of *TP53*-mutated clones. Mutations in *TP53* are present in the skin but provide a survival advantage only in response to chronic UV light exposure.⁷⁷ Similarly, *TP53* mutations in the intestine have a clonal advantage only in the setting of chronic inflammation,⁷⁸ and clonal expansion of *TP53* is observed in patients with irritable bowel disease⁷⁹ but has not been recorded in the healthy colon mucosa. It is tempting to speculate that similar mechanisms may be at play in SDS or del (5q) MDS,⁸⁰ in which leukemic transformation is often associated with *TP53* mutations.^{43,81} Genotoxic stress in the SDS environment led to cell-cycle checkpoint activation and apoptosis in normal HSPCs.²⁸ It is conceivable that mutations dampening the cell-cycle checkpoint may lead to competitive survival of mutated clones, perhaps at the expense of accelerated mutagenesis, a hypothesis that awaits experimental definition.

However, inflammation driving expansion of resistant clones, of relevance to human MDS, is beginning to be documented in the case of *TET2* mutations. *Tet2*-knockout murine and *TET2*-mutant human HSPCs have a clonal advantage caused by resistance to apoptosis in an in vitro environment that contains the pro-inflammatory cytokine tumor necrosis factor- α (*TNF α*).⁸² *TET2*-deficient macrophages are hyperinflammatory,⁸³ and this may exacerbate processes by propagating an inflammatory environment.^{67,84} Furthermore, increased IL-6 production or activation of TLR2 is critical for the development of the myelo-proliferative phenotype of *TET2*-deficient hematopoietic cells.⁸⁵

Together, the data point to necessary contributions of the aberrant (inflammatory) microenvironment to drive the induction, selection, and eventual expansion of mutant clones that are poised for subsequent malignant transformation (Figure 1). The data strongly argue that MDS needs to be considered a disease of a tissue rather than a disease of hematopoietic cells in isolation. Cross talk between HSPCs and their (mesenchymal) environment likely drives disease initiation and evolution. This has important consequences for our thinking about prognostication and treatment of this disease. Niche parameters should be considered complementing HSPC-intrinsic characteristics in future models of prognostication, and targeting environmental contributions (eg, by dampening inflammation through inhibition of TLR4 signaling, ROS,²⁸ or the NLRP3 inflammasome^{39,67}) may not only ameliorate BM failure but ultimately attenuate or prevent leukemic evolution if the selective pressure for poisoning clones can be alleviated.

Acknowledgment

This work was supported by Dutch Cancer Society (KWF Kankerbestrijding) grants EMCR 2016-10488 and 2017-11092.

Authorship

Contribution: E.P. wrote the article, provided literature background, and assisted in illustrating. M.H.G.P.R. wrote the article and supervised E.P.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Footnote

Submitted 1 October 2018; accepted 6 November 2018. Prepublished online as *Blood* First Edition paper, 22 January 2019; DOI 10.1182/blood-2018-10-844639.

REFERENCES

1. Woll PS, Kjällquist U, Chowdhury O, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo [published corrections appear in *Cancer Cell*. 2014;25(6):861 and 2015; 27(4):603-605]. *Cancer Cell*. 2014;25(6):794-808.
2. Sperleng AS, Gibson CJ, Ebert BL. The genetics of myelodysplastic syndrome:

- from clonal haematopoiesis to secondary leukaemia. *Nat Rev Cancer*. 2017;17(1):5-19.
3. Abdel-Wahab O, Gao J, Adli M, et al. Deletion of *Asx1* results in myelodysplasia and severe developmental defects in vivo. *J Exp Med*. 2013;210(12):2641-2659.
4. Mupo A, Seiler M, Sathiaselan V, et al. Hemopoietic-specific *Sf3b1-K700E* knock-in mice display the splicing defect seen in human

MDS but develop anemia without ring sideroblasts. *Leukemia*. 2017;31(3):720-727.

5. Shirai CL, Ley JN, White BS, et al. Mutant *U2AF1* expression alters hematopoiesis and pre-mRNA splicing in vivo. *Cancer Cell*. 2015; 27(5):631-643.
6. Kim E, Ilagan JO, Liang Y, et al. SRSF2 mutations contribute to myelodysplasia by mutant-specific effects on exon recognition. *Cancer Cell*. 2015;27(5):617-630.

7. Weidner H, Rauner M, Trautmann F, et al. Myelodysplastic syndromes and bone loss in mice and men. *Leukemia*. 2017;31(4):1003-1007.
8. Mellibovsky L, Diez A, Serrano S, et al. Bone remodeling alterations in myelodysplastic syndrome. *Bone*. 1996;19(4):401-405.
9. Raaijmakers MH. Myelodysplastic syndromes: revisiting the role of the bone marrow microenvironment in disease pathogenesis. *Int J Hematol*. 2012;95(1):17-25.
10. Li AJ, Calvi LM. The microenvironment in myelodysplastic syndromes: Niche-mediated disease initiation and progression. *Exp Hematol*. 2017;55:3-18.
11. Kerbauy DM, Lesnikov V, Torok-Storb B, Bryant E, Deeg HJ. Engraftment of distinct clonal MDS-derived hematopoietic precursors in NOD/SCID-beta2-microglobulin-deficient mice after intramedullary transplantation of hematopoietic and stromal cells. *Blood*. 2004;104(7):2202-2203.
12. Flynn CM, Kaufman DS. Donor cell leukemia: insight into cancer stem cells and the stem cell niche. *Blood*. 2007;109(7):2688-2692.
13. Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature*. 2014;505(7483):327-334.
14. Wei Q, Frenette PS. Niches for hematopoietic stem cells and their progeny. *Immunity*. 2018;48(4):632-648.
15. Raaijmakers MH, Mukherjee S, Guo S, et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. *Nature*. 2010;464(7290):852-857.
16. Walkley CR, Olsen GH, Dworkin S, et al. A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. *Cell*. 2007;129(6):1097-1110.
17. Rupec RA, Jundt F, Rebholz B, et al. Stroma-mediated dysregulation of myelopoiesis in mice lacking I kappa B alpha. *Immunity*. 2005;22(4):479-491.
18. Kode A, Manavalan JS, Mosialou I, et al. Leukaemogenesis induced by an activating beta-catenin mutation in osteoblasts. *Nature*. 2014;506(7487):240-244.
19. Dong L, Yu WM, Zheng H, et al. Leukaemogenic effects of Ptpn11 activating mutations in the stem cell microenvironment. *Nature*. 2016;539(7628):304-308.
20. Xiao P, Dolinska M, Sandhow L, et al. *Sipa1* deficiency-induced bone marrow niche alterations lead to the initiation of myeloproliferative neoplasm. *Blood Adv*. 2018;2(5):534-548.
21. Medyouf H, Mossner M, Jann JC, et al. Myelodysplastic cells in patients reprogram mesenchymal stromal cells to establish a transplantable stem cell niche disease unit. *Cell Stem Cell*. 2014;14(6):824-837.
22. Balderman SR, Li AJ, Hoffman CM, et al. Targeting of the bone marrow microenvironment improves outcome in a murine model of myelodysplastic syndrome. *Blood*. 2016;127(5):616-625.
23. Schepers K, Pietras EM, Reynaud D, et al. Myeloproliferative neoplasia remodels the endosteal bone marrow niche into a self-reinforcing leukemic niche. *Cell Stem Cell*. 2013;13(3):285-299.
24. Arranz L, Sánchez-Aguilera A, Martín-Pérez D, et al. Neuropathy of haematopoietic stem cell niche is essential for myeloproliferative neoplasms. *Nature*. 2014;512(7512):78-81.
25. Duarte D, Hawkins ED, Akinduro O, et al. Inhibition of endosteal vascular niche remodeling rescues hematopoietic stem cell loss in AML. *Cell Stem Cell*. 2018;22(1):64-77.e6.
26. Boyd AL, Reid JC, Salci KR, et al. Acute myeloid leukaemia disrupts endogenous myelo-erythropoiesis by compromising the adipocyte bone marrow niche. *Nat Cell Biol*. 2017;19(11):1336-1347.
27. Mead AJ, Neo WH, Barkas N, et al. Niche-mediated depletion of the normal hematopoietic stem cell reservoir by Flt3-ITD-induced myeloproliferation. *J Exp Med*. 2017;214(7):2005-2021.
28. Zambetti NA, Chen S, Ping Z, et al. Mesenchymal inflammation drives genotoxic stress in hematopoietic stem cells and predicts disease evolution in human pre-leukemia. *Cell Stem Cell*. 2016;19(5):613-627.
29. Chen S, Zambetti NA, Bindels EMJ, et al. Massive parallel RNA sequencing of highly purified mesenchymal elements in low-risk MDS reveals tissue-context-dependent activation of inflammatory programs. *Leukemia*. 2016;30(9):1938-1942.
30. Feng X, Scheinberg P, Wu CO, et al. Cytokine signature profiles in acquired aplastic anemia and myelodysplastic syndromes. *Haematologica*. 2011;96(4):602-606.
31. Iancu-Rubin C, Mosoyan G, Wang J, Kraus T, Sung V, Hoffman R. Stromal cell-mediated inhibition of erythropoiesis can be attenuated by Sotatercept (ACE-011), an activin receptor type II ligand trap. *Exp Hematol*. 2013;41(2):155-166.e17.
32. Ping Z, Chen S, Hermans SJF, et al. Activation of NF-κB driven inflammatory programs in mesenchymal elements attenuates hematopoiesis in low-risk myelodysplastic syndromes [published online ahead of print 12 October 2018]. *Leukemia*. doi:10.1038/s41375-018-0267-x.
33. Wang L, Zhang H, Rodriguez S, et al. Notch-dependent repression of miR-155 in the bone marrow niche regulates hematopoiesis in an NF-κB-dependent manner. *Cell Stem Cell*. 2014;15(1):51-65.
34. DiDonato JA, Mercurio F, Karin M. NF-κB and the link between inflammation and cancer. *Immunol Rev*. 2012;246(1):379-400.
35. Pikarsky E, Porat RM, Stein I, et al. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature*. 2004;431(7007):461-466.
36. Vogl T, Eisenblätter M, Völler T, et al. Alarmin S100A8/S100A9 as a biomarker for molecular imaging of local inflammatory activity. *Nat Commun*. 2014;5(1):4593.
37. Flores-Figueroa E, Varma S, Montgomery K, Greenberg PL, Gratzinger D. Distinctive contact between CD34+ hematopoietic progenitors and CXCL12+ CD271+ mesenchymal stromal cells in benign and myelodysplastic bone marrow. *Lab Invest*. 2012;92(9):1330-1341.
38. Schneider RK, Schenone M, Ferreira MV, et al. Rps14 haploinsufficiency causes a block in erythroid differentiation mediated by S100A8 and S100A9. *Nat Med*. 2016;22(3):288-297.
39. Basiorka AA, McGraw KL, Eksioglu EA, et al. The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. *Blood*. 2016;128(25):2960-2975.
40. Lane SW, Sykes SM, Al-Shahrour F, et al. The *Apc*(min) mouse has altered hematopoietic stem cell function and provides a model for MPD/MDS. *Blood*. 2010;115(17):3489-3497.
41. Wang J, Fernald AA, Anastasi J, Le Beau MM, Qian Z. Haploinsufficiency of *Apc* leads to ineffective hematopoiesis. *Blood*. 2010;115(17):3481-3488.
42. Stoddart A, Wang J, Hu C, et al. Inhibition of WNT signaling in the bone marrow niche prevents the development of MDS in the *Apc^{del/+}* MDS mouse model. *Blood*. 2017;129(22):2959-2970.
43. Stoddart A, Wang J, Fernald AA, Karrison T, Anastasi J, Le Beau MM. Cell intrinsic and extrinsic factors synergize in mice with haploinsufficiency for *Tp53*, and two human *del(5q)* genes, *Egr1* and *Apc*. *Blood*. 2014;123(2):228-238.
44. Bhagat TD, Chen S, Bartenstein M, et al. Epigenetically aberrant stroma in MDS propagates disease via Wnt/β-catenin activation. *Cancer Res*. 2017;77(18):4846-4857.
45. Johnson RC, Greenberg PL, Gratzinger D. CD271+ mesenchymal stromal cell density is high in poor-risk MDS and independently predicts overall survival [abstract]. *Blood*. 2013;122(21). Abstract 1560.
46. Zhao Y, Wu D, Fei C, et al. Down-regulation of *Dicer1* promotes cellular senescence and decreases the differentiation and stem cell-supporting capacities of mesenchymal stromal cells in patients with myelodysplastic syndrome. *Haematologica*. 2015;100(2):194-204.
47. Fei C, Zhao Y, Guo J, Gu S, Li X, Chang C. Senescence of bone marrow mesenchymal stromal cells is accompanied by activation of p53/p21 pathway in myelodysplastic syndromes. *Eur J Haematol*. 2014;93(6):476-486.
48. Geyh S, Oz S, Cadeddu RP, et al. Insufficient stromal support in MDS results from molecular and functional deficits of mesenchymal stromal cells. *Leukemia*. 2013;27(9):1841-1851.
49. Vallée A, Lecarpentier Y. Crosstalk between peroxisome proliferator-activated receptor gamma and the canonical WNT/β-catenin pathway in chronic inflammation and oxidative stress during carcinogenesis. *Front Immunol*. 2018;9:745.
50. Chien Y, Scuoppo C, Wang X, et al. Control of the senescence-associated secretory phenotype by NF-κB promotes senescence and enhances chemosensitivity. *Genes Dev*. 2011;25(20):2125-2136.
51. Crescenzi E, Pacifico F, Lavorgna A, et al. NF-κB-dependent cytokine secretion controls Fas expression on chemotherapy-induced

- premature senescent tumor cells. *Oncogene*. 2011;30(24):2707-2717.
52. Rovillain E, Mansfield L, Caetano C, et al. Activation of nuclear factor-kappa B signalling promotes cellular senescence. *Oncogene*. 2011;30(20):2356-2366.
53. Geyh S, Rodríguez-Paredes M, Jäger P, et al. Transforming growth factor β -mediated functional inhibition of mesenchymal stromal cells in myelodysplastic syndromes and acute myeloid leukemia. *Haematologica*. 2018; 103(9):1462-1471.
54. Coppé J-P, Desprez P-Y, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol*. 2010;5(1):99-118.
55. Park D, Spencer JA, Koh BI, et al. Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. *Cell Stem Cell*. 2012;10(3): 259-272.
56. Lopez-Villar O, Garcia JL, Sanchez-Guijo FM, et al. Both expanded and uncultured mesenchymal stem cells from MDS patients are genomically abnormal, showing a specific genetic profile for the 5q- syndrome. *Leukemia*. 2009;23(4):664-672.
57. Ramakrishnan A, Awaya N, Bryant E, Torok-Storb B. The stromal component of the marrow microenvironment is not derived from the malignant clone in MDS. *Blood*. 2006;108(2): 772-773.
58. Flores-Figueroa E, Arana-Trejo RM, Gutiérrez-Espindola G, Pérez-Cabrera A, Mayani H. Mesenchymal stem cells in myelodysplastic syndromes: phenotypic and cytogenetic characterization. *Leuk Res*. 2005;29(2): 215-224.
59. Blau O, Hofmann WK, Baldus CD, et al. Chromosomal aberrations in bone marrow mesenchymal stroma cells from patients with myelodysplastic syndrome and acute myeloblastic leukemia. *Exp Hematol*. 2007;35(2): 221-229.
60. Migliaccio AR. A vicious interplay between genetic and environmental insults in the etiology of blood cancers. *Exp Hematol*. 2018; 59:9-13.
61. Lo WJ, Lin CL, Chang YC, et al. Total body irradiation tremendously impair the proliferation, differentiation and chromosomal integrity of bone marrow-derived mesenchymal stromal stem cells. *Ann Hematol*. 2018; 97(4):697-707.
62. Si S, Nakajima-Takagi Y, Iga T, et al. Hematopoietic insults damage bone marrow niche by activating p53 in vascular endothelial cells. *Exp Hematol*. 2018;63:41-51.e1.
63. Asumda FZ. Age-associated changes in the ecological niche: implications for mesenchymal stem cell aging. *Stem Cell Res Ther*. 2013; 4(3):47.
64. Franceschi C, Garagnani P, Vitale G, Capri M, Salvioi S. Inflammaging and 'Garb-aging'. *Trends Endocrinol Metab*. 2017;28(3): 199-212.
65. Zhang DY, Wang HJ, Tan YZ. Wnt/ β -catenin signaling induces the aging of mesenchymal stem cells through the DNA damage response and the p53/p21 pathway. *PLoS One*. 2011;6(6):e21397.
66. Swindell WR, Johnston A, Xing X, et al. Robust shifts in S100a9 expression with aging: a novel mechanism for chronic inflammation. *Sci Rep*. 2013;3(1):1215.
67. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*. 2017;355(6327): 842-847.
68. Ribezzo F, Snoeren IAM, Ziegler S, et al. Rps14, Csnk1a1 and miRNA145/miRNA146a deficiency cooperate in the clinical phenotype and activation of the innate immune system in the 5q- syndrome [published online ahead of print 19 January 2019]. *Leukemia*. doi: 10.1038/s41375-018-0350-3.
69. Vas V, Senger K, Dörr K, Niebel A, Geiger H. Aging of the microenvironment influences clonality in hematopoiesis. *PLoS One*. 2012; 7(8):e42080.
70. McCullough KD, Coleman WB, Smith GJ, Grisham JW. Age-dependent induction of hepatic tumor regression by the tissue microenvironment after transplantation of neoplastically transformed rat liver epithelial cells into the liver. *Cancer Res*. 1997;57(9): 1807-1813.
71. Liggett LA, DeGregori J. Changing mutational and adaptive landscapes and the genesis of cancer. *Biochimica et biophysica acta. Reviews on cancer*. 2017;1867(2):84-94.
72. Marusyk A, Casás-Selves M, Henry CJ, et al. Irradiation alters selection for oncogenic mutations in hematopoietic progenitors. *Cancer Res*. 2009;69(18):7262-7269.
73. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature*. 2015;518(7540):552-555.
74. Coombs CC, Zehir A, Devlin SM, et al. Therapy-related clonal hematopoiesis in patients with non-hematologic cancers is common and associated with adverse clinical outcomes. *Cell Stem Cell*. 2017;21(3): 374-382.e4.
75. Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci USA*. 2001;98(21):12072-12077.
76. Henry CJ, Casás-Selves M, Kim J, et al. Aging-associated inflammation promotes selection for adaptive oncogenic events in B cell progenitors. *J Clin Invest*. 2015;125(12): 4666-4680.
77. Klein AM, Brash DE, Jones PH, Simons BD. Stochastic fate of p53-mutant epidermal progenitor cells is tilted toward proliferation by UV B during preneoplasia. *Proc Natl Acad Sci USA*. 2010;107(1):270-275.
78. Vermeulen L, Morrissey E, van der Heijden M, et al. Defining stem cell dynamics in models of intestinal tumor initiation. *Science*. 2013; 342(6161):995-998.
79. Galandiuk S, Rodríguez-Justo M, Jeffery R, et al. Field cancerization in the intestinal epithelium of patients with Crohn's ileocolitis. *Gastroenterology*. 2012;142(4):855-864.e8.
80. Mei Y, Zhao B, Basiorka AA, et al. Age-related inflammatory bone marrow microenvironment induces ineffective erythropoiesis mimicking del(5q) MDS. *Leukemia*. 2018;32(4): 1023-1033.
81. Xia J, Miller CA, Baty J, et al. Somatic mutations and clonal hematopoiesis in congenital neutropenia. *Blood*. 2018;131(4): 408-416.
82. Abegunde SO, Buckstein R, Wells RA, Rauh MJ. An inflammatory environment containing TNF α favors Tet2-mutant clonal hematopoiesis. *Exp Hematol*. 2018;59:60-65.
83. Cull AH, Snetsinger B, Buckstein R, Wells RA, Rauh MJ. Tet2 restrains inflammatory gene expression in macrophages. *Exp Hematol*. 2017;55:56-70.e13.
84. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017; 377(2):111-121.
85. Meisel M, Hinterleitner R, Pacis A, et al. Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. *Nature*. 2018;557(7706):580-584.