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REVIEW

Stem cell origin of myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) are common hematologic disorders that are characterized by decreased blood counts due to ineffective hematopoiesis. MDS is considered a 'preleukemic' disorder linked to a significantly elevated risk of developing an overt acute leukemia. Cytopenias can be observed in all three myeloid lineages suggesting the involvement of multipotent, immature hematopoietic cells in the pathophysiology of this disease. Recent studies using murine models of MDS as well as primary patient-derived bone marrow samples have provided direct evidence that the most immature, self-renewing hematopoietic stem cells (HSC), as well as lineage-committed progenitor cells, are critically altered in patients with MDS. Besides significant changes in the number and distribution of stem as well as immature progenitor cells, genetic and epigenetic aberrations have been identified, which confer functional changes to these aberrant stem cells, impairing their ability to proliferate and differentiate. Most importantly, aberrant stem cells can persist and further expand after treatment, even upon transient achievement of clinical complete remission, pointing to a critical role of these cells in disease relapse. Ongoing preclinical and clinical studies are particularly focusing on the precise molecular and functional characterization of aberrant MDS stem cells in response to therapy, with the goal to develop stem cell-targeted strategies for therapy and disease monitoring that will allow for achievement of longer-lasting remissions in MDS.

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INTRODUCTION

Malignant transformation occurs as a step-wise process in which genetic and epigenetic aberrations accumulate and lead to progressive alteration of the molecular makeup and function of cells.[1–3] If initially formed 'pre-malignant' cells are not cleared by the organism, further alterations may ultimately generate fully transformed cells that initiate the cancer, so-called cancer stem cells. As the acquisition of mutations is not a rapid process, it is reasonable to assume that pre-malignant alterations occur either in cells with a long life span, such as tissue-specific stem cells, or the initial mutation has to confer self-renewal ability to a cell with a shorter life span. Recent findings have shown that cancer stem cells can exist as pools of relatively quiescent and long-lived cells that do not respond well to common cell-toxic agents and thereby contribute to disease relapse.[4] Myeloid malignancies also arise from a population of aberrant cells that in most cases are not eliminated by conventional cytotoxic therapies, and which share many cellular features with normal hematopoietic stem cells, such as sustained self-maintenance, proliferative capacity, and some degree of differentiation potential.[5] Various murine models of acute myeloid leukemia have demonstrated that these aberrant cells, capable of initiating the leukemia in lethally irradiated recipient mice (leukemia-initiating cells) can reside within the population of phenotypical hematopoietic stem cells (HSC)[6–9] but also among lineage-committed progenitor cells.[10–12] The exact molecular mechanism which ultimately leads to malignant transformation of immature stem and myeloid progenitor cells is, however, still not understood. Myelodysplastic syndromes (MDS) are considered a 'preleukemic' disease that is characterized by massively impaired hematopoiesis with decreased peripheral blood counts that can affect all blood lineages. While it has been assumed for a long time that MDS is a 'stem cell disease', only recently reports have provided hard evidence for this hypothesis and have described profound alterations in rigorously defined stem and progenitor cells of MDS patients.[13–15] In addition to quantitative abnormalities, recent findings have revealed molecular alterations in MDS stem cells and demonstrated the persistence of these cells in relapse.[15] As patients with MDS have a significant risk of developing acute myeloid leukemia, understanding the molecular and functional aberrations in hematopoietic stem and progenitor compartments in MDS will not only enable the development of more efficient and durable treatment options, but may also provide valuable insights into the transformation process of acute myeloid leukemias.

MDS-INITIATING CELLS RESIDE IN THE HSC COMPARTMENT, HARBOR CYTOGENETIC ALTERATIONS AND SUPPRESS COEXISTING RESIDUAL NORMAL HSC

Early studies characterizing immature hematopoietic cells in patients with MDS have focused on patients with recurring chromosomal aberrations using fluorescence *in-situ* hybridization in stem and progenitor cell-enriched compartments.[16] The advent of multi-parameter FACS has enabled studies on more precisely defined stem cells, which in combination with fluorescence *in-situ*

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hybridization analysis allow for the separation of normal HSC and stem cells being part of the aberrant clone. Recent studies on patients with −5q aberrations showed that while only a minority of pro-B and pro-T cells were cytogentically abnormal, the majority of myeloid cells and 95–98% of $CD34^+CD38^-CD90^+$ (Thy1) HSC had the cytogenetic defect. Functional studies *ex vivo* furthermore indicated that the $CD34^+CD38^-$ HSC compartment harbors disease-relevant cells with 5q deletions.[17] A study of MDS patients with trisomy of chromosome 8 showed that this cytogenetic alteration was not as frequently seen in the $CD34^+CD38^-$ HSC.[18] Furthermore, examination of patients that had both trisomy 8 and 5q− abnormalities showed that only del(5q) occurred in the more primitive stem cell compartments, suggesting that several clones of aberrant cells organized in a hierarchical fashion reside in the bone marrow of these patients (for overview see Table 1). Our own studies on highly purified stem and progenitor populations from MDS patients with −7/7q-revealed that a very high proportion of precisely defined HSC ($Lin^-CD34^+CD38^-CD90^+$) and progenitors contained the −7/7q-abnormality (>90%) while unfractionated whole bone marrow cells only showed a mean involvement of 60% of karyoptypically abnormal cells, demonstrating that these alterations are strikingly enriched in stem and progenitor cells in MDS.[15] Interestingly, B and T lymphocytes in MDS patients rarely contain these abnormalities,[19–27] demonstrating that normal HSC clones must still be present from which lymphocytic cells can originate, and that aberrations occurring in MDS HSC are incompatible with the commitment and differentiation to the lymphoid lineages. Our understanding of the cellular and molecular basis of how MDS HSC gain a growth advantage over residual normal stem cells is still incomplete. Thus, future studies have to focus on elucidating the mechanisms by which abnormal MDS stem cells dominate normal HSC in order to effectively treat the disease and prevent the progression to acute leukemia.

QUANTITATIVE AND FUNCTIONAL CHANGES IN STEM AND PROGENITOR COMPARTMENTS IN MDS

Besides the presence of cytogenetically abnormal cells within the stem cell compartment of patients with MDS, quantitative changes within the stem and immature progenitor compartments of patients with MDS have been reported, revealing compromised hematopoiesis at several stages of immature hematopoietic differentiation in these patients. Interestingly, the well-described clinical heterogeneity of the disease is already reflected at the stem and progenitor cell level as we and others have found alterations in the abundance of different immature hematopoietic stem and progenitor compartments that were specific to certain MDS subtypes,[13,15,28] suggesting that subgroups of MDS share common pathophysiologic characteristics. Reduced MEP populations have been observed almost universally in all subtypes of MDS, pointing to a differentiation block or apoptosis prior to this stage, and are in line with the decreased numbers of erythrocytes and platelets, a hallmark of this disease. Several other stem and progenitor cell compartments show subtype-restricted alterations. The bone marrow of patients with lower risk MDS was characterized by a specific expansion of the phenotypic CMP compartment and a relative reduction of MEP and GMP populations, possibly pointing to a differentiation block at this cellular level, while phenotypical HSC varied in frequency more than age-matched controls.[13,29] One recent study has attributed the reduction in the GMP population seen in lower risk MDS to an enhanced apoptotic cascade with concomitant upregulation of the prophagocytic marker calreticulin, which was found to be specific to the myeloid progenitor compartment.[13] An expansion of the entire $Lin^-CD34^+CD38^-$ stem cell compartment was observed within higher risk subtypes of MDS.[15,28] This expansion was most pronounced in phenotypically primitive LT-HSC ($Lin^-CD34^+CD38^-CD90^+$). Patients with higher risk MDS also showed a varying yet significant expansion of the GMP compartment with concomitant expression of the antiphagocytic marker CD47, and a relative decrease of the MEP compartment[13] reminiscent of the alterations in myeloid progenitor population distribution seen in AML.[5,29] The observation of specific progenitor expansions raise the possibility that these expanded progenitor populations might have a disease-initiating ability. Even though these studies have not been conducted in human MDS, murine models of AML have shown that phenotypic GMP contain the disease-initiating cells.[30] Further studies that test this hypothesis in human MDS and AML are needed and may reveal important

Table 1. Hierarchical organization of cells with cytogenetic aberrations in MDS

Cytogenetic aberration	*Hematopoietic cell compartment analyzed*	*Frequency of cells with cytogenetic aberration (% average and SEM)*			*Study*
5q−	$CD34^+CD38^-$	98 (1.0)			Nilsson *et al.*[17]
	$CD34^+CD38^+$	96 (1.2)			
	$CD15^+$	86 (5.3)			
	$CD19^+$	8 (2.8)			
	$CD3^+$	3 (0.6)			
+8	$CD34^+CD38^-Thy1^+$	62 (10)			Nilsson *et al.*[18]
	$CD34^+CD38^-Thy1^-$	71 (8)			
	$CD34^+CD38^-$	56 (7.7)			
	$CD34^+CD38^+$	55 (9.5)			
	$CD34^-$	34 (8.8)			
+8, 5q− case report		+8	5q−	+8, 5q−	
	$CD34^+CD38^-$	0	34	66	
	$CD34^+CD38^+$	0	80	20	
	Granulocytic	0	81	17	
	Erythroid	0	82	6	
5q−	$CD34^+CD38^-Thy1^+$	97 (0.8)			Nilsson *et al.*[42]
	$CD34^+CD38^+$	98 (0.7)			
−7	$Lin^-CD34^+CD38^-Thy1^+$	95 (3.3)			Will *et al.*[15]
	$Lin^-CD34^+CD38^-Thy1^-$	88 (5.2)			
	CMP	85 (5.6)			
	GMP	96 (1.8)			
	MEP	91 (5.6)			
	Whole BM	62 (4.4)			

insights into the contribution of disease-relevant immature hematopoietic compartments other than stem cells in the context of disease evolution and progression.

MOLECULAR ALTERATIONS IN MDS STEM AND PROGENITOR CELLS

Several studies, which aimed to elucidate the molecular underpinnings of MDS, have found significant alterations in the genetic, transcriptional and epigenetic makeup of aberrant cells at several differentiation stages. Due to limitations dictated by available technology, many past studies have characterized either unfractionated bone marrow cells or $CD34^+$ cell-enriched populations. Given the significant alterations in stem and progenitor frequencies, differences at the molecular level determined by these studies reflect to a certain point alterations in stem and progenitor cell composition and largely mask underlying disease-relevant alterations. Molecular screening techniques for the detection of mutations, differential gene expression, as well as epigenetic alterations have been constantly refined in the past few years and now enable the comprehensive study of very limited numbers of cells. The continued dissection of the precise stage and subtype-specific molecular alterations in MDS stem and progenitor cells will provide further insight into the evolution of this disease as well as its progression to AML and will reveal targets for specific therapy, which is urgently desired.

Genetic alterations

Studies investigating the mutational status of precisely defined MDS stem and progenitor cells are currently lacking. However, besides well-described karyotypic changes in MDS[31] several recent studies investigating total bone marrow and $CD34^+$ cells have identified recurrent mutations, including in genes encoding signaling molecules,[1,2] transcriptional,[2] epigenetic[7–9,32] and pre-mRNA splicing factors.[10,12] A recent comprehensive study by Bejar and colleagues demonstrated that somatic point mutations are common in unfractionated total bone marrow-derived cells in MDS and that >50% of patients carry at least one somatic mutation.[2] They also identified a set of 18 commonly mutated genes, some of which were found associated with specific clinical characteristics. Particularly, mutations in *TP53, EZH2, ETV6, RUNX1* and *ASXL1* have been identified as independent predictors of poor overall survival in patients with MDS,[2] showing that certain smaller genetic lesions can contribute to the disease pathogenesis. These findings suggest that the extensive cellular heterogeneity hallmarked by a variable presence and severity of cytopenias, aberrant blast cells as well as great differences in the patients' response to treatment, their progression to overt AML, and ultimately survival, is at least in part driven by various combinations of genetic aberrations and different cellular compartments these alterations occur in. To further understand the molecular origin of MDS, future investigations will have to address at which cellular level and in which order these somatic mutations occur, how the disruption of particular genes contribute to the formation, maintenance and the gain of growth advantage of MDS HSC, as well as their contribution to disease relapse.

Transcriptional changes

Several studies have utilized transcriptome profiling with gene expression arrays to elucidate the differences between immature hematopoietic cells in MDS in comparison to healthy controls. Early studies were performed on the heterogeneous population of marrow-derived $CD34^+$ cells from MDS patients and identified MDS-associated gene expression profiles in immature hematopoietic cells.[33–36] Deregulated transcriptional programs were reported for the three commonest MDS cytogenetic subtypes (del(5q), trisomy 8 and −7/del(7q)), with Wnt/β-catenin signaling, integrin signaling and cell cycle regulation as the predominant deregulated pathways.[37] In addition, transcriptional changes in $CD34^+$ cells associated with specific FAB subtypes were reported, including interferon-stimulated genes *IFIT4, IFIT1* and *IFITM1* showing increased expression.[36] This finding also hints at the important role that cytokines have in the regulation of hematopoietic stem cell self-renewal[38] and, particularly, in bone marrow dysplasia and aplasia syndromes,[39–41] and which orchestrate apoptotic effects on erythroid progenitor cells, resulting in the pathognomonic refractory anemia. $CD34^+$ cells of MDS patients with trisomy of chromosome 8 were characterized by deregulation of genes related to immune and inflammatory response such as *TGFBR3, IGF-1 and MAF-1*. $CD34^+$ cells from MDS cases with deletions affecting chromosome 7 showed deregulated signaling pathways such as SAPK/JNK, PI3K/AKT and NF-κB, suggesting enhanced survival mechanisms, and thus worse outcomes in this group.[37]

Profiling of more highly enriched HSC ($CD34^+CD38^-CD90^+$) from MDS patients with 5q− showed that critical regulators of self-renewal, such as BMI1 and Delta-like homolog were upregulated; potentially explaining the growth potential with poor hematopoietic multilineage differentiation capacity of 5q− $CD34^+CD38^-CD90^+$ cells.[42] This study also identified the stage-specific downregulation of CCAAT enhancer binding protein-alpha in MDS progenitors ($CD34^+CD38^+CD90^-$), a gene critical for normal myeloid development and known to be involved in the transformation of cells in AML. The 5q deleted region showed resultant gene dosage effect (downregulation) with reduced levels of *TAF7, CDC42SE2* and *CTNNA1* in $CD34^+CD38^-CD90^+$ cells. Our own group has recently carried out and reported a gene expression analysis using rigorously purified HSC ($Lin^-CD34^+CD38^-CD90^+$) from patients with MDS and age-matched healthy controls, and in conjunction with epigenetic profiling,[15] which is discussed in the next paragraph.

Epigenetic alterations

In addition to transcriptomic changes, epigenetic changes have been found in MDS stem cells. Methylome analysis of $CD34^+$ cells from MDS patients with the HELP assay revealed widespread hypermethylation in MDS samples. Among the genes aberrantly hypermethylated in MDS when compared with normal $CD34^+$ cells were those from the WNT signaling pathway, including *GSK3β*, *APC2, SFRP1, SFRP2, SFRP3, FRAT1*and *AXIN2*, as well as genes encoding for *Wnt* proteins 2b, 3, 4, 5, 6, 9, 10 and 16.[43] We have recently examined the transcriptome and methylome of highly purified HSC ($Lin^-CD34^+CD38^-CD90^+$) from MDS patients.[15] We utilized an optimized version of the HpaII tiny fragment enrichment by ligation-mediated PCR (HELP) assay that could work with nanogram amounts of DNA from sorted cells (nano-HELP assay).[44] In contrast to studies done on whole bone marrow that found predominant hypermethylation in MDS, our study of rigorously sorted HSC shows both aberrant hypo- and hypermethylated loci in MDS. We found aberrant methylation of several genes not previously linked to MDS pathobiology, such as hypermethylated- *NOTCH4, RET* and *DLL3*, and hypomethylated *NOTCH1, NANOG* and *HDAC4* in MDS stem cells, thereby providing the first evidence of epigenetic alterations in the earliest definable HSC. These findings suggest that specific target genes are genetically and epigenetically deregulated in early stem and progenitor cells in MDS, which may make these cells therapeutically targetable. Despite considerable inter-sample variability, 9 genes were consistently hypomethylated and overexpressed in MDS-HSC *(STAT3, WDR5, OBFC2B, SKA3, HEXA, CIAPIN1, VRK3, CHAF1B* and *RANBP1)*. Functional studies of one such target, STAT3, showed that inhibition of STAT3 activation by

small molecule inhibitors led to an 82% inhibition of the clonogenic capacity of Lin $CD34^+CD38^-$ of MDS stem cells while age-matched healthy donor purified HSC were not significantly affected.[15] Taken together, these studies show that MDS HSC harbor epigenetic, transcriptional and karyotypic alterations, which warrant further study in larger clinical cohorts and can be leveraged for the development of targeted, MDS stem cell-directed therapies in the future.

Immunophenotypical alterations of MDS HSC

In order to precisely understand the molecular mechanism by which MDS stem cells dominate normal stem cells and evade current therapies, further characterization of highly purified aberrant and normal stem and progenitor cells are required. Recent and current efforts have focused on the discovery of cell surface marker genes, allowing for the prospective isolation of viable aberrant stem and progenitor cells and subsequently for their functional and molecular characterization. A recent study has identified the glycoprotein CD99 as a distinct surface marker present on MDS HSCs. The expression of CD99 was found predominantly in the $CD34^+CD38^-$ fraction of stem cells in MDS/AML, and $CD99^+$ expressing HSC demonstrated greater colony forming capacity and leukemia initiating cell activity in xenografts. On the contrary, CD99 low expressing colonies showed phenotypic properties resembling normal HSC and MPP and lacked molecular abnormalities.[45] Work from our own laboratory has shown that the surface protein IL1RAP is overexpressed on fractionated HSC from patients with high-risk MDS, but not low-risk MDS,[46] and may thus represent a novel marker of MDS disease progression at the stem cell level. However, the exact functional relevance of aberrant expression of CD99 and IL1RAP on stem and multipotent progenitors patients with MDS/AML still needs to be determined. Deciphering the cellular and molecular mechanisms that separate aberrant from normal or predisposed stem and progenitor cells will provide novel therapeutic targets for the specific elimination of all transformed, disease-relevant cells.

PRE-LEUKEMIC STEM CELLS IN MDS AND AML

Recent findings in patients including at the minimal residual disease stage and in patients at relapse after achieving complete remission have brought into perspective the existence of pre-leukemic clones in AML, a feature that is likely shared with MDS.[28,47,48] Several studies in AML mouse models have shown strong evidence of mutations in transcription factors to be early events in leukemic clonal evolution.[11,49,50] Some of these mutants impair normal gene function and disrupt hematopoiesis by increasing the self-renewing capacity of myeloid progenitors, with targeted expansion of the MPP compartment, prior to progression of overt leukemia, such as AML1-ETO and MLL-AF9.[51,52] Simultaneously, murine models with diminished expression of key transcription factors *(sfi1, Junb, Gata1&2, Hlx, HoxB4, HoxA9, TGF-β, Wnt* and *Notch)* have shown to perpetuate increased HSC numbers, which evade immunological response and are propagated in the niche, primed to undergo subsequent leukemic transformation.[6,53–62] However, the precise mechanisms that lead to dysregulated expression of key transcription factors in murine and human leukemogenesis are still incompletely understood. Recent work has identified crucial epigenetic regulatory factors that influence the expansion and differentiation of HSC and progenitors. Many gene loss-of-function murine models (*Tet2*, *IDH1&2*, *EZH2*, *Dnmt1&3a*, *Asxl1*)[3,63–69] have revealed augmented HSC numbers, skewed myeloid-specific expansion and myelodysplastic features but none of these develop frank leukemia. Recent work has shown that some of these disease alleles cooperate in the initiation of MDS.[70,71] Deregulated metabolic pathways in murine HSC have also shown to contribute towards the origin of a pre-LSC and leukemogenesis. Loss of genes such as *Pten*, *Pml, Fbxw7, Hif-1β, Lkb1*[72–77] leads to expansion of the HSC compartment, with gradual development of a myeloproliferative disorder. Taken together, clinical and mouse data have provided strong evidence for the existence of pre-LSC populations, which are involved in disease initiation and also in disease relapse, thus warranting future studies to ultimately permit targeting of these heterogeneous and functionally critical cellular subsets.

ABERRANT HSC AND DISEASE RELAPSE IN MDS

The role of MDS HSC in disease relapse has been most extensively studied in 5q− syndrome. Lenalidomide is an immunomodulatory drug that has been shown to induce hematologic remissions in 67% of patients with 5q− syndrome and is FDA approved for this disease. Unfortunately, there is a high rate of relapse after this treatment. Recently, in del(5q) MDS, rare stem cells, selectively resistant to Lenalidomide were shown to persist at the time of complete clinical and cytogenetic remission.[14] Lenalidomide was more effective in reducing the del(5q) clone in $CD34^+CD38^+$ progenitors than in $CD34^+CD38^{-/low}CD90^+$ (constituting $<0.5\%$ of bone marrow mononuclear cells) stem cells. This provided evidence of the selective persistence of the phenotypically distinct population of $CD34^+CD38^{-/low}CD90^+$ stem cells in del(5q) MDS in patients with a high relapse rate. Also, a large fraction of $CD34^+CD38^{-/low}CD90^+$ stem cells, unresponsive to lenalidomide, were found to be quiescent, predominantly in the G0 phase and continued to show no response to cytokine induction. Furthermore, the expression of several genes linked to stem-cell function and leukemic transformation (*HOXA9, MEIS1, MCL1, BMI1, C-MYC* and *MYCN*)[14,78–81] and several efflux pumps (ABCB1, ABCB7, and ABCC1) were upregulated in del(5q) $CD34^+CD38^{-/low}CD90^+$ stem cells, possibly contributing to their drug-resistant properties.[81,82] These cells also exhibited a significant upregulation of cell-surface IL3RA at the time of remission as well as during progression.[14]

Our own studies have recently evaluated the role of karyotypically abnormal stem cells during treatment with 5-azacytidine, an agent that is FDA approved for treatment of MDS.[15] Serial examination of bone marrow obtained from a patient treated with a combination of 5-azacytidine and vorinostat revealed the high-level persistence of karyotypically abnormal HSC with the chromosome 7 deletion even at the time of complete morphological remission with normal blood counts and dramatic reduction of −7 cells in unfractionated bone marrow cells. Expansion of clonotypic HSC preceded a fulminant clinical relapse by two months, strongly suggesting that relapse is driven out of the aberrant stem cell compartment and that the analysis at the stem cell level has diagnostic and prognostic value. These findings provide a cellular explanation of the high relapse rate in high-risk MDS patients treated with epigenetic modifiers such as HDAC and DNMT inhibitors, suggesting that these agents do not lead to the eradication of clonally abnormal HSC in MDS, even upon a very good morphological remission and hematologic recovery.

Another recent study[83] also evaluated the impact of epigenetic modifying drugs 5′-azacitidine and valproate on leukemic stem/progenitor populations, especially LMPP-like ($Lin^-CD34^+CD38^-CD90^-CD45RA^+$) and GMP-like ($CD34^+CD38^+CD123^{+/low}BAH\text{-}1^-CD45RA^+$) cells in patients with high-risk AML and MDS. The authors observed abnormal expansions in these immunophenotypically distinct LSC populations, as high as 12-fold when compared with normal controls after three cycles of treatment, despite morphologic responses and normalization in marrow blast counts. These expansions predated frank morphological relapses as long as eight months. Partial and

non-responders showed negligible reduction in their LSC populations, again strongly suggesting the critical importance of these stem cell compartments for initial therapeutic response and relapse rates.

STEM CELL ALTERATIONS IN MURINE MODELS OF MYELODYSPLASIA

Several murine models of myelodysplasia have been reported, mainly generated via transgenic expression of mutant genes or deletions of genes involved in epigenetic regulation, ribosomal homeostasis or maintenance of marrow microenvironment. These models are characterized by dysplastic differentiation, increased rates of leukemic transformation and cytopenias. Examination of stem and progenitor cells in these mice reveals both quantitative and qualitative defects that can point to stem cell alterations that are potentially relevant in human disease (for overview see Table 2).

The commonly deleted region linked with the 5q- syndrome lies within chr5q31 and 5q32 and contains various candidate genes, including the ribosomal protein *RPS14* as well as *miR-145* and *miR-146a*. Haploinsufficiency of *RPS14* was shown to lead to decreased erythropoiesis in an *in-vitro* RNAi screen[84] and was then modeled in mice. A mouse model with *CD74-Nid67* deletion on chromosome 18, which also included the *RPS14* gene[85] displays a phenotype consistent with MDS and develops macrocytic anemia. These mice demonstrate impaired progenitor cell production in the BM (reduced CFU-E, CFU-GM, CFU-MK populations) and have reduced absolute cell numbers of HSC, CMP and GMP in both BM and spleen, which is at least in part due to increased rates of *p53*-mediated apoptosis. Increased rates of stem and progenitor cell apoptosis mimic findings in human 5q-syndrome. *miR-145* and *miR-146a* are expressed abundantly in normal hematopoietic stem and progenitor cells where they repress Toll-interleukin-1 receptor domain-containing adaptor protein and tumor necrosis factor receptor-associated factor-6, respectively. These two non-coding RNAs were recently identified to be lost in bone marrow cells of patients with 5q − MDS,[31,86] with *miR-145* also playing a role in response to treatment. Importantly, in a murine model, loss of *miR-145* and *miR-146a* and overexpression of tumor necrosis factor receptor-associated factor-6 phenocopied several clinical features of 5q − MDS,[31] and targeting of the associated *interleukin-1 receptor-associated kinase 1* was recently reported as a promising therapeutic approach in MDS.[87] *Nucleophosmin1* (*NPM1*), is another gene located in the 5q region (despite not being located in the commonly deleted region) and encodes for a nucleolar protein that is known to modulate ribosomal biogenesis and centrosome duplication. While *NPM1* mutations are amongst the most frequent genetic alterations in AML,[88,89] *NPM1* is mutated in $<5\%$ of MDS patients.[90] Hypomorphic $Npm1^{+/-}$ mice were reported to contain amplified immature erythroblastic populations with increased karytotypic abnormalities (tetraploidy) and dysplastic megakaryopoeisis and granulopoesis, thus resembling clinical subsets of MDS.[91,92]

The deletion of *Dicer1,* an endonuclease essential for microRNA biogenesis, in osteolineage-specific progenitors in the marrow, was reported to lead to the development of leukopenias, alongside variable anemia and thrombocytopenia.[93] Reduced production of the erythrocytic and megakaryocytic lineages in the bone marrow was largely compensated by splenic extra-medullary hematopoiesis. No significant differences in the frequency or functional impairment of immunophenotypically defined HSC (LSK-SLAM) or progenitor cells derived from $Dicer^{flox/flox}$ and $Dicer^{flox/+}$ bone marrow was found. However, increased apoptosis was observed in megakaryocyte-erythroid progenitors. The propagation of a myelodysplastic phenotype was governed by immature cells of the osteolineage, in that stromal cells with osterix$^+$ osteocalcin$^-$ expression were able to induce dysplastic features *in vivo*. On the contrary, *Dicer* gene-ablated terminally differentiated osteoblasts (osteocalcin$^+$) could not recapitulate the hematological abnormalities. Dysplastic changes in the marrow of $Dicer^{flox/flox}$ mice were noted as early as 3 weeks, and they further developed myeloid sarcomas and acute monocytic leukemia-like disease. This proposes that a defunct 'marrow-niche' could be the site for a series of events preceding the evolution of a leukemic clone, possibly through its influence on immature hematopoietic cells.

Mutations in the *Ten-Eleven Translocation-2* (*TET2*) gene have been described in MDS and AML patients.[32,88,89] The *TET2* gene is involved in hydroxylation of 5-methylcytosine (5-mc),[94,95] thereby modulating epigenetic patterns. While *TET2* mutations with a corresponding decrease in 5-hydroxy methylcytosine have been frequently detected in humans with MDS, MPD, and AML,[32,89] conditional *Tet2* KO models were reported to give rise to a CMML-like disease.[68] Analysis of the marrow of these mice showed increased replating potential of LSK-CD150$^+$ HSC with an upregulation of myeloid progenitor markers CD34$^+$FCγR$^+$ and increased expression of self-renewal genes including *Meis1* and *Evi1*. Quantitatively, these mice demonstrated an expansion of their HSC (LSK-SLAM) and progenitor (Lin$^-$c-kit$^+$CD34$^+$FCγR$^+$) populations in the spleen, but not in the BM. Importantly, bone marrow-derived cells from $Tet2^{-/-}$ mice had enhanced competitive repopulating capacity in comparison with WT when transplanted and followed-up at 23 weeks. Another recent study[96] carried out a phenotypic characterization of two models, one which was similar to the previous conditional KO model, and the second being a 'gene-trap' *Tet2* KO with 20–50% residual expression of *Tet2*. In addition to many features described in the previous model, the gene trap model showed a differentiation arrest in the erythroid lineage both in the BM and spleen as well as an expansion of immature aberrant B cell compartments and an immature T-cell (CD44$^+$CD25$^-$CD4$^-$CD8$^-$) population. Thus, the inactivation of *Tet2* may also alter T- and B-cell differentiation in mice.

Mutations in metabolic enzymes such as *IDH1* and *IDH2* have been found in AML and MDS and are mutually exclusive with *TET2* mutants.[3] Mutants of IDH1 (R132) and IDH2 (R140/R172),[7,97–99] acquire a neo-catalytic function with production of 2-hydroxy-glutarate, an analog of α-ketoglutarate (α-KG).[100,101] This oncogenic metabolite impairs the catalytic function of α-KG-dependent enzymes, including TET2, thereby reversibly decreasing the levels of 5-hydroxymethylcytosine (5-hmc) hmc and leading to DNA hypermethylation and the induction of leukemia.[101] *Idh1* (R132H) knock-in transgenic mice generated by a myeloid-promoter (LysM), have been shown to lead to a preferential expansion of Lin$^-$ Sca^{-1+}cKit$^+$ cells (LSK) while sparing myeloid progenitor populations (CMP, GMP, MEP) in the BM and spleen of older compared with younger mice.[102] Moreover, the splenic cells had greater propensity to form myeloid colonies than BM cells, suggestive of extra-medullary hematopoiesis, a feature resulting from a dysfunctional marrow niche and pathognomonic of MDS. Extension of these findings in transgenic mice driven by the Vav promoter, showed constitutive expression of the mutation in all hematopoietic cells and further expansion of the aforementioned populations as well as progenitors (including lymphoid progenitors), suggestive of an incomplete differentiation block. Transgenic LSK cells showed 80% increased methylation of CpG, including at loci involved in signaling pathways, hematopoietic differentiation, and leukemic stem cell maintenance. Taken together, these findings demonstrate that *IDH1/2* mutations are possibly acquired as early events in an evolving primitive hematopoietic cell, initiating acquisition of key epigenetic changes favoring leukemogenesis and full transformation. Given their role in leukemogenesis, mutant *IDH1/2* is currently explored as a novel therapeutic target in AML and glioblastoma.[103–105]

Ectopic viral integration 1 (*EVI1*), a nuclear transcription factor and proto-oncogene has been shown to regulate hematopoietic

Table 2. Overview of previous studies investigating the function of hematopoietic stem and progenitor cells purified from patients with myelodysplastic syndrome by xenotransplantation

Gene	*Mouse model*	*Defect*	*Flow cytometry characteristics*
Npm (Grisendi *et al.*,[91] Sportoletti *et al.*[92])	*Npm1*$^{+/-}$	Genomic instability Centrosome duplication Upregulation of p53	Bone marrow: Increase in TER119^{hi}CD71^{hi} (immature erythroblastic precursor markers) and tetraploidy. 5–10% of CFU-E/BFU-E showed dysplastic features. After a 2-year f/u, the mature myeloid population (Gr-1 and Mac-1) had doubled when compared with control (*Npm1*$^{+/+}$).
Rps14 (Barlow *et al.*[85])	Deletion of 5q CDR	Defective ribosomal biogenesis. Increased induction of p53.	Severe reduction in BM hematopoietic progenitor colonies (CFU-E, CFU-GM, CFU-MK). HSC (IL-7Rα^- Lin$^-$c-kit^{high}Sca-1$^-$)/ LSK-SLAM, CMP-MEP (Lin$^-$c-kit^{high}Sca-1$^-$CD16/CD32^{low}) and GMP (Lin$^-$c-kit^{high}Sca-1$^-$CD16/CD32^{high}) progenitor cells were depleted in BM and spleen.
Dicer1 (Raaijmakers *et al.*[93])	Transgenic	Disrupting microRNA biogenesis. Altering hematopoietic stem cell niche. Sbds gene repressed.	No change in frequency or functionality of HSC (LSK CD150$^+$CD48$^-$). Apoptosis was most pronounced in the MEP. Mutation had reduced B-cell and corresponding progenitors.
Tet2 (Moran-Crusio *et al.*[68])	Conditional KO (Tet2$^{f/f}$)	Impaired 5-hydroxymethylation of the transcriptome.	BM of *Tet2* KO mice failed to show any gross changes in stem (LSK-SLAM) or progenitor population (Lin$^-$c-kit$^+$CD34$^+$FCγR$^+$) albeit in spleen cells both these populations showed statistically significant vs WT (10% vs 5% and 12% vs 2%, respectively). *Tet2* KO cells show increased repopulating ability vs WT after competitive transplantation in BM at 23 weeks in LT-HSC (89% vs9%), MPP1 (95% vs 3%), MPP2 (98% vs 1%), CMP (96% vs 3%), myeloid mature (94% vs 3%) and lymphoid mature (96% vs 4%). *Tet2* haplosufficiency is sufficient to replicate the *Tet2* KO phenotype albeit is able display slower repopulating kinetics.
Tet2 (Quivoron *et al.*[96])	Conditional KO (Tet2$^{f/f}$) Gene trap Tet2$^{LacZ/LacZ}$ (50–80% reduction)	Impaired 5-hydroxymethylation of the transcriptome. Possible role of Tet1 in aiding transformation.	BM from both *Tet2*$^{-/-}$ models showed an increase in LSK fraction within this population ST-HSC (Lin$^-$Sca-1$^+$c-Kit$^+$CD34$^+$Flt3$^-$) and to a lesser extent LT-HSC (Lin$^-$Sca-1$^+$c-Kit$^+$CD34$^-$ Flt3$^-$). Myeloid progenitor cells, especially CMP and MEP populations, further showed an absolute increase. Peripheral blood and spleen showed a marked amplification in mature myeloid population (CD11b$^+$Gr1$^-$). Erythrocytic lineage showed a differentiation arrest in the spleen and BM, as the population of proerythroblasts (CD71$^+$Ter119$^+$) superseded that of late erythroblasts (CD71^{lo} Ter119$^+$). Lymphoid lineage reflected similar results with an increase only in the immature T-cell thymic population (CD4$^-$CD8$^-$), while B-cell lineage showed a depleted absolute count.
Idh1 (Figueroa *et al.*,[3,65] Sasaki *et al.*[102])	Transgenic Idh1 (R132H) mutants [LysM-KI/Vav-KI]	By-product of Idh1 mutant 2HG, an analog of α-KG, inhibits enzymatic activity of TET2. Resulting in global hypermethylation.	Lin$^-$ BM/splenic cells showed a 5-fold increase in the LSK population, predominantly the LT-HSC (CD150$^+$CD48$^-$) and multipotent progenitor population (CD150$^-$CD48$^+$), of older versus younger mice, while there was no alteration in the progenitor populations (CMP, GMP, CLP, MEP). Moreover, KI splenic cells showed enhanced myeloid-CFC assays when compared with BM samples (within normal limits). Repopulation KI-BM assays showed no diminished capacity at 170 days post-transplantation.
Evi1 (Buonamici *et al.*,[107] Laricchia-Robbio *et al.*,[108] Louz *et al.*[109])	Transgenic	Transcriptional repression of *EpoR* and *c-Mpl*. Inactivating the transcriptional activity of GATA1 and 2. Impaired response to Epo. Inhibits TGF-β signaling. Enhances E2F activity by repressing BRG1.	Evi1 overexpression results in a depletion of the CFU-E colonies. Spleens and BMs showed increased expression of Ter119$^+$ in *Evi1-Tg* vs control mice (41.8 vs 21.2%).

Table 2. (Continued)

Gene	*Mouse model*	*Defect*	*Flow cytometry characteristics*
Nup98-Hox (Choi *et al.*,[116] Lin *et al.*[117])	Transgenic mice and ES cell line NUP98-HOXD13	Differentiation blockade by upregulation of MYB. Requires additional genetic events for leukemogenesis.	NHD13 Lin^{neg} BMMNC with mutation showed a decrease in colony formation (total = 13 ± 6, CFU-GM = 4 ± 5, CFU-GEMM = 2 ± 3) vs WT. With SCF, IL-3, IL-6 supplementation, NHD13 vs WT Lin^{neg} BMMNC (29.2 ± 1.6% vs 3.1 ± 0.9%) at 7 days.
Sall4B (Ma *et al.*[118])	Transgenic (SALL4B)	Activates Wnt-β catenin signaling.	Samples from BM, spleen, LN and CFU (day 7) showed positive expression for CD45 and myeloid markers such as Gr-1 and Mac-1 (81.9%). However, an increase in immature cells was observed in the transgenic group as identified by c-kit (10.2%), $CD34^+$ (14.8%) and increase in myeloid specific colonies (CFU-GM = 54%).

Shown are utilized recipient mouse strains (granulocyte/monocyte colony-stimulating factor (GM-CSF), steel factor (SF), interleukin-3 (IL-3)), route of transplantation (intravenous (IV), intraperitoneal (IP)), population type and number of transplanted cells per mouse (bone marrow (BM), biopsy (Bx), bone marrow mononuclear cells (BMMNC), mesenchymal stem cells (MSC), myelodysplastic syndrome (MDS), acute myeloid leukemia with myelodysplasia-related changes (AML-MRC)), as well as outcome (chimerism, expressed as percent (%) engraftment and % clonal engraftment; myeloid and lymphoid lineage outputs) (Data not available (NA)).

homeostasis as well as disrupt normal cell differentiation *in vitro*.[106] Moreover, Sca-1 promoter-driven *Evi1* transgenic mice[107–109] showed a predominant reduction in the erythroid lineage with a decrease in CFU-E colonies, and an expansion in immature erythroid precursors in the BM and spleen albeit circulating RBC were within normal limits and other lineages were unaffected. The underlying mechanism has been attributed majorly to the repression in transcriptional activity of *GATA1* and *GATA2* with resultant decreased responsiveness to erythropoeitin.[110,111] Ectopic expression of *Evi1* also resulted in susceptibility toward developing myeloid leukemia in newborn mice.[109]

Nucleoporin (*NUP98*) translocations have been associated with some cases of MDS and therapy-related MDS (1–2%).[112] Physiologically, NUP98 acts as a docking site for 'karyopherins', a group of nuclear transport signal receptor proteins, and regulates the nucleo-cytoplasmic shuttling of proteins and RNA.[113] NUP98 fusion partners in MDS or AML include *HOXD11* and *HOXD13*.[114,115] *Nup98-Hoxd13* (*Nhd13*) transgenic mice[116,117] develop a fulminant MDS-like disease with transformation into acute leukemia and mortality within 14 months. Lin^- bone marrow nucleated cells from *Nhd13* mice showed a severe reduction in CFU-GM and CFU-GEMM colony formation when compared with WT Lin^- bone marrow nucleated cells. Functional studies demonstrated impaired differentiation from Lin^- cells in *Nhd13* mice when compared with WT controls. Interestingly, a higher percentage of apoptotic cells was detected by flow analysis within the *Nhd13* Lin^- bone marrow nucleated cells in culture when supplemented with cytokines (IL-3, IL-6, SCF), consistent with the view that these cells have an impaired ability to differentiate and undergo apoptosis when exposed to differentiating cytokines.

MDS has been associated with *SALL4B* overexpression and increased *SALL4* expression is also seen in up to 60% of M1 and M2 subtypes of AML.[118] Transgenic mice with *Sall4* overexpression[118] developed MDS-phenotype in 6–8 months and 50% of cases progressed towards AML. Bone marrow, spleen and lymph node (LN) samples showed an increased positivity for $c\text{-}kit^+$ progenitor cells and a higher expression of myeloid markers such as Gr-1 and Mac-1.

Even though these mouse models have shown stem cell dysfunction and other features consistent with MDS, most models are still limited by incomplete penetrance, mixed MPD/MDS phenotypes and highly variable rates of disease penetrance and transformation to acute leukemia.

MODELING OF HUMAN MDS

Primary MDS stem cells have shown reduced xenografting capabilities in several studies (for overview see Table 3). Early studies with $CD34^+CD38^-$ cell populations from patients with 5q deletion demonstrated engraftment from only 1 out of 5 MDS patients. The sample that engrafted, involved transplantation of 700 000 $CD34^+$ cells and showed as much as 12% human $CD45^+$ engraftment.[17] Similar studies conducted by the same group in MDS patients with the trisomy 8 defect have shown no engraftment in 17 mice transplanted with $CD34^+$, $CD34^+$ $CD38^-$ or $CD34^+$ $CD38^+$ cells.[18] Low proportions of engrafted cells were also observed in a larger study that transplanted marrow-derived MDS cells into NOD/SCID-$\beta2m^{null}$ mice.[119] The engraftment observed was also short lived and mainly myeloid with very few human lymphoid cells. Transplantation in NOD/SCID-$\beta2m^{null}$ mice engineered to produce human interleukin-3, granulocyte-macrophage colony-stimulating factor and stem cell factor resulted in worse engraftment, suggesting that lack of these human cytokines was not the reason for poor xenotransplantation potential of MDS cells. A modification of this approach was used by Deeg and Coworkers[120] who injected a total of 10 million whole BMMC derived from 6 MDS patients into the femurs of NOD/SCID-$\beta2m^{null}$ mice along with 1×10^5 cells each of human stromal-derived cell lines (HS5 and HS27a), previously well characterized.[121,122] Of the 15 mice injected, 11 showed evidence of engraftment. 95% of cells in all but 1 of the 6 mice showed evidence of clonal precursors, which included del(5q), del(7q) and loss of Y chromosome, and at high frequencies which had not been previously demonstrated. The higher rates of clonal cells were attributed not only to the intrafemoral route of transplantation but also to the co-injection of human stromal cells that provide crucial auxiliary signals facilitating engraftment.

A recent study[123] reported that intramedullary injection of bone marrow $CD34^+$ hematopoietic cells obtained from patients with MDS or AML-MRC (AML with MDS-related changes) into the bone marrow of NOG mice with human MSC improved engraftment of human cells in the murine microenvironment. Lineage analysis revealed a $CD33^+$ myeloid dominant differentiation in half of the MDS cases and up to 40% of the AML-MRC cases, suggesting the engraftment of MDS-originated cells. This engraftment was also significantly enhanced by the auxiliary delivery of MSC, as mice transplanted with bone marrow $CD34^+$ and MSC expressed a greater fraction of $CD33^+$ cells than controls. In contrast,

Table 3. Xenograft studies using MDS stem cells

Study	*Recipient strain*	*Conditioning of mice*		*Route*	*Transplanted cell compartment*	*Number of cells injected*	*Engraftment of total human cells*	*Engraftment of clonal MDS cells*	*Cell lineage engraftment*		*Long-term engraftment*
		Irradiation (cGy)	*Auxiliary cells?*						*Myeloid*	*Lymphoid*	
Nilsson *et al.*[17]	8–12 week old NOD/LtSz-SCID	350	Yes	IV	$CD34^+$ $CD34^+$ $CD38^-$	7×10^5 $6–17 \times 10^3$	1/7 mice 12% $hCD45^+$	100% cells with 5q del	NA	NA	NA
Nilsson *et al.*[18]	8–12 week old NOD/SCID NOD/SCIDß2m$^{-/-}$	350–375	Yes	IV	$CD34^+$ $CD34^+CD38^-$ $CD34^+$ $CD38^+$	$0.2–2.5 \times 10^6$ 17.5– 2500×10^3 $0.2–2.5 \times 10^6$	0/17 mice	No karyotypically abnormal cells.	NA	NA	NA
Benito *et al.*[128]	6–8 week old NOD/SCID	300–375	No	IV IP SC	BM $CD34^+$ BM $CD34^+$ Bx $CD34^+$	1×10^7 2.0×10^7 0.60– 0.75×10^7	62.5% of mice 62.5% of mice 85.7% of mice	0.5 − 14%.	Yes	Yes	Yes (normal) No (MDS)
Thanopoulou *et al.*[119]	8–10 week old NOD/SCIDß2m^{null} (expressing human IL-3/GM-CSF/SF) NOD/SCIDß2m^{null}	350	Yes	IV IV	Lin^- Lin^-	$4–17 \times 10^6$ $4–17 \times 10^6$	11/16 mice 20/27 mice	Detectable +8 trisomy in 0.6–2.7% of cells del(5q) 85% in samples from 1 patient	70 ± 10% (@15–23 weeks) 1.5–5.2%	13 ± 7% (@15–23 weeks) NA	5/11 @15–23 weeks 7/20 15–23 weeks
Kerbauy *et al.*[120]	NOD/SCIDß2m^{null}	350	Yes	IM	10^7 whole BMMCs	BMMC1 $\times 10^7$	$CD45^+$ 11/15 mice (0.7–58.4%). Most in the spleen followed by PB. This proportion was consistent throughout the 17-week f/u.	5/6 in mice f/u from 4–17 weeks BM: 9.8-50% Spleen: 6.4–10%.	NA	NA	Up to 17 weeks
Muguruma *et al.*[123]	NOD/SCID/IL2Rγ^{null} (NOG)	300	Yes	IM	$CD34^+$	14– 1000×10^4	$CD45^+$ MDS group 8/8 mice. AML-MRC group 12/23 mice.	100% cells in 5/6 mice	$CD33^+$ >60% cells. (>80% when MSCs were auxiliary cells @ 8–16 weeks)	@ 8–16 weeks $CD19^+$ ~29.1%	Up to 16 weeks
Li *et al.*[129]	Nod.cg-Prkdc*scid* Il2rg^{tm1wjll} (NSG)	300	Yes	IM	$CD34^+$ PB-MNC	5×10^6 10×10^6	100% of mice engrafted 0.2–18.39% $hCD45^+$ in BM 0–73.2% $hCD45^+$ in SP	22–33% in BM 4–11% in SP	Yes	Yes	Up to 13 weeks

Abbreviations: AML-MRC, acute myeloid leukemia with myelodysplasia related changes; BM, bone marrow; BMMC, bone marrow mononuclear cells; Bx, biopsy; GM-CSF, granulocyte monocytic-colony stimulating factor; IM, intramedullary; IP, intraperitoneal; IV, intravenous; MDS, myelodysplastic syndrome; MSC, mesenchymal stem cells; NA, not available; PB, peripheral blood; SC, sub cutaneous; SF, steel factor.

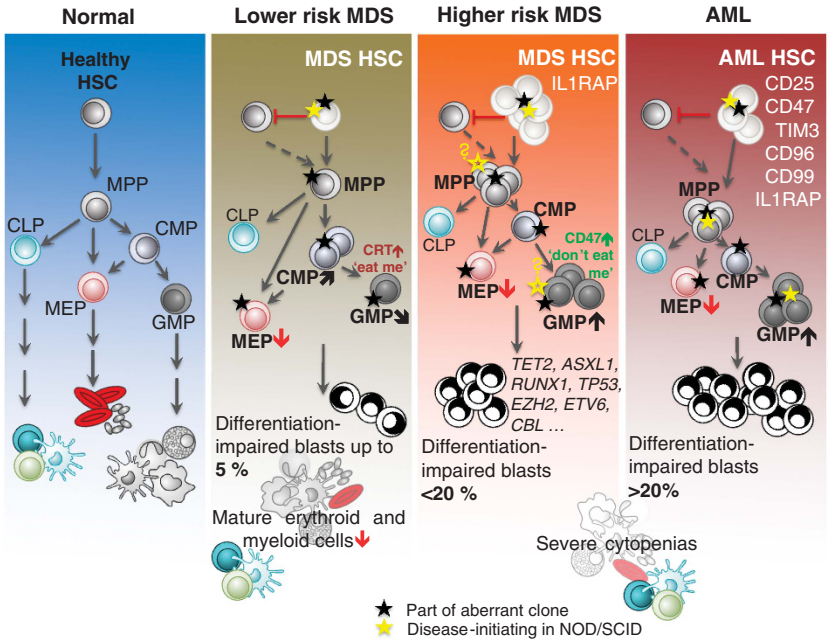

Figure 1. Overview of cellular and molecular aberrations occurring in patients with MDS and AML in comparison to normal immature hematopoietic cells. The hematopoietic stem cell (HSC) compartment in patients with lower-risk and higher-risk MDS contains aberrant cells (indicated by the solid black star) with phenotypical alterations (such as overexpression of IL1RAP, CD25, CD47, TIM3, CD96 and CD99) and show functional changes (indicated by the solid yellow star), which lead to the suppression of healthy HSC (solid cell) and the generation of aberrant multipotent progenitor cells (MPP). Disease-reinitiating cells, as tested in adoptive cell transfer studies utilizing immunocompromized mice, can be contained in the stem and progenitor compartments in AML patients. Whether disease-initiating cells are also residing in the progenitor cell compartment in MDS remains to be formally shown (open yellow star with question mark). Aberrant multipotent progenitor cells give rise to myeloid lineage-committed cells showing further phenotypical as well as functional changes. While in lower-risk MDS a significant reduction of the megakaryocytic-erythroid progenitor (MEP) compartment is frequently observed along with a mild reduction of granulocytic-monocytic progenitor cells (GMP) and increased common myeloid progenitors (CMP), MDS patients of the higher-risk group, similar to patients with AML, present with a significantly expanded GMP and HSC compartments, while the MEP population is severely reduced. Mechanistically, the reduction of the GMP compartment has been linked to an elevated expression of a prophagocytic marker (CRT, 'eat me') which results in their programmed cell removal. In contrast, the increase of GMP in higher-risk MDS patients has been demonstrated to be at least partially mediated through a process allowing for the evasion of phagocytosis due to upregulation of an antiphagocytic marker (CD47, 'don't eat me'). Together, while these cellular and molecular changes are in line with the observed cytopenias and emergence of aberrant blast cells in the bone marrow and peripheral blood of patients with MDS and AML, further studies are required to determine the order of occurrence and cooperation of multiple molecular events, and the precise cellular compartments affected during the multi-step transformation process.

mice transplanted with cells obtained from healthy individuals showed a predominant engraftment of B-lineage cells, as earlier documented in NOG mice.[124–126] Cytology and bone marrow histology of the xenografted mice revealed that the marrow compartment was filled with large human CD45$^+$ leukemic blasts. Particularly, the endosteal region of the marrow was enriched with human CD34$^+$ cells, indicating an invasion of hematopoietic stem cell niches by MDS-originated CD34$^+$ cells.

A study from our own laboratory used NOD-SCID IL2 receptor gamma null mice and found engraftment of BM MNC from a high-risk MDS patient, and that this model could be utilized for preclinical drug testing.[127] Finally, a recent study has shown that FACS-sorted MDS HSC can engraft irradiated, newborn NOD-SCID IL2 receptor gamma null recipient mice transplanted with as few as 1000 purified HSC.[13] The engraftment in these mice was shown to last >12 weeks and resulted predominantly in myeloid progeny. Karyotypic analysis revealed a small but consistent number of aberrant cells, suggestive of no competitive disadvantage of MDS HSC in the xenotransplantation assay. Altogether, these studies show that MDS stem cells do not engraft very well in murine models, though it is possible that the use of more immunodeficient or younger mice may enhance xenografting abilities of MDS cells. These, and even more refined models which are currently being developed, will greatly enhance our ability to study human MDS (stem) cells in the preclinical setting, determine their susceptibility to existing and new treatments, and identify new compounds and drug combinations that are effective at the level of disease-initiating (stem) cells and which may lead to a longer lasting control of the disease.

CONCLUSION

Much of our previous understanding of MDS stem cells was based on and inferred from studies in AML. Utilizing both genetically engineered mouse models as well as patients with human MDS, recent work has provided clear evidence of stage-specific quantitative and qualitative, cell-autonomous alterations of HSC and progenitors in MDS (for overview see Figure 1). Even though human MDS stem cells are still hard to xenograft, newer immuno-deficient mice and novel transplantation strategies have been developed, which are instrumental in the molecular and functional study of MDS stem cells. Translational studies have shown that clonally abnormal stem cells can survive during clinical remissions and can predict relapses following standard therapies. It will be critical to continue to study these disease-driving cell populations, and to develop strategies to specifically target them in order to achieve lasting remissions and possibly a cure of the disease in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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