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Mouse models of myelodysplastic syndromes

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Synopsis

Three general approaches have been used in an attempt to model myelodysplastic syndrome (MDS) in mice, including treatment with mutagens or carcinogens, xenotransplantation of human MDS cells, and genetic engineering of mouse hematopoietic cells. Xenotransplantation of cells from MDS patients has proved difficult, possibly due to the innate characteristics of the MDS clone and microenvironmental influences, including adverse effects of a host immune response. Genetic engineering of hematopoietic cells or mice has been accomplished by *in vitro* transfer of genes to mouse hematopoietic cells with subsequent transplantation into an irradiated host, or by modification of the mouse germline to generate mice with altered expression of genes of interest. A number of genes have been studied using these approaches, including *RUNX1*, *Evi1*, *Npm1*, *SALL4*, *NUP98-HOXD13*, *BCL2/NRAS*, *Arid4a*, *Polg* and *Dido*. This review discusses the phenotypes observed in available mouse models for MDS with a concentration on a model that leads to aberrant expression of conserved homeobox (HOX) genes that are important regulators of normal hematopoiesis. Utilizing these models of MDS should allow a more complete understanding of the disease process and provide a platform for pre-clinical testing of therapeutic approaches.

Keywords

myelodysplastic syndrome; mouse model; xenotransplant; genetically engineered mice; Hoxa

Mouse models of cancer

A wide spectrum of model organisms has been used to investigate the biology of cancer. These model organisms include *Drosophila melanogaster*, *Danio rerio*, *Rattus* (several species), *Mus musculus*, and non-human primates. For a variety of reasons, including small body size, high fecundity, well characterized physiology, and completely sequenced genome, the common laboratory mouse (*Mus musculus*) has been the model organism of choice for many cancer biologists. Three principal strategies have been employed to model cancer in the mouse. Mice

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have been treated with known mutagenic or carcinogenic agents, most commonly small molecules 1:2 or viruses³. In addition, immunodeficient mice have been used in xenotransplant experiments in which primary human malignancies or cell lines are injected into the mouse. Finally, recently developed molecular genetic tools have made possible the generation of genetically engineered mice (GEM). All three of these approaches have been used in an attempt to model myelodysplastic syndrome (MDS) in mice.

Nomenclature for hematopoietic disease in mice has been the topic of considerable debate. The hematopathology subcommittee of the Mouse Models of Human Cancer Consortium has developed a set of guidelines for the uniform description of hematopoietic malignancies in mice⁴. These criteria are presented in Table 1. It should be noted that most malignancies fulfilling the criterion for “cytopenia with increased blasts” are classified as acute non-lymphoid leukemias, and not myeloid dysplasia. A mouse that fulfills criteria 1 (cytopenias) and 2B (increased BM blasts), along with splenomegaly and disseminated disease (as reflected by invasion of parenchymal tissues or > 20% circulating blasts) are classified as ANLL, not MDS. Dyspoiesis in mice can be difficult to identify. Megaloblastic changes and multinucleate erythroblasts are signs of dysplasia in the erythroid lineage that are similar in both mice and humans, but ringed sideroblasts are rare in mice. Micromegakaryocytes, large megakaryocytes with unlobated nuclei, and large megakaryocytes with bizarre hypersegmentation are signs of megakaryocytic dysplasia in mice. Neutrophil dyspoiesis may be evident in the form of pseudo-Pelger-Huet cells with fine nuclear bridging⁵ and cells with lobated, as opposed to ring nuclei.

Xenotransplantation

Immunodeficient mouse models bearing human tumor xenografts have been established for the study of solid tumor biology^{4,6,7}, as well as for the study of hematopoietic malignancies and identifications of leukemia-initiating cells^{8–10}. Although the xenograft model is an attractive approach for investigating malignant disease, many human tumors are unable to be established in the mouse host. This could be due to a variety of reasons, including toxicity from *ex vivo* manipulation of the malignant cells, a lack of necessary growth/survival factors in the tumor microenvironment, or an anti-tumor immune response generated by the host. Recently, immunodeficient mouse models for the study of leukemia-initiating cells have been subjects of careful scrutiny for the effects of the host immune system on xenografts, specifically sorted primary leukemia cells¹¹. In that study, the authors demonstrated that flow cytometric sorting of leukemic cells with a CD38 antibody led to an Fc-mediated clearance of the sorted leukemia cells *in vivo*. Some of these same challenges may have hindered efforts to establish xenograft models of MDS (perhaps to a further extent).

Several labs have attempted to engraft immortal human cell lines established from MDS patients. Until proven otherwise, by recapitulating MDS in a host, these cell lines should not be regarded as MDS cell lines, but rather as AML cell lines that have evolved, *in vivo* or *in vitro*, from MDS patients¹². In order to provide the growth factors that would increase the likelihood of engraftment of F-36P, a cell line derived from a patient with MDS, *Scid* (severe combined immunodeficient mice) mice were engineered to express the human forms of GM-CSF (granulocyte macrophage-colony stimulating factor) and IL-3 (interleukin-3)¹³. Despite the proliferation of F-36P in the transgenic mice, engraftment was only achieved following neutralization of NK cells. These mice also developed osteolytic lesions, a rare occurrence in MDS patients.

More recently, the non-obese diabetic (NOD)/ SCID mouse has been employed in xenograft studies of primary cells from MDS patients, since these mice are defective in complement and NK cell activities, in addition to B and T cell deficiencies. In one study, hematopoietic cells from 7 MDS patients with a 5q deletion were injected into NOD/SCID mice. One of the seven

mice showed evidence for low level (12%) engraftment, and while CD45+CD15+ cells contained the 5q deletion, no evidence for clinical disease was found in the recipient mice ¹⁴. Similarly, bone marrow from MDS patients with trisomy 8 was unable to reconstitute lymphoid and myeloid compartments in mice ¹⁵. In a second study, bone marrow from patients with MDS was injected into sublethally irradiated NOD/SCID mice, with or without the delivery of human cytokines ¹⁶. While subfractions of human CD45+ cells (*e.g.* CD34-CD38+ and T cells) were detected in mice transplanted with MDS bone marrow, decreased percentages of cells from the MDS patients engrafted as compared with bone marrow from healthy controls. Additionally, abnormal karyotypes detected in bone marrow from MDS patients (*e.g.* del(5q)) prior to the transfer of cells to mice were not identified after transplantation, suggesting that the majority of the human cells that had engrafted were derived from normal bone marrow elements, as opposed to the MDS clone. The authors attribute the lack of observed engraftment of clonal MDS cells to several possibilities, including decreased proliferative potential, adverse microenvironmental conditions, and susceptibility to immune attack.

In an effort to evade immune responses, as well as provide human cytokines in the context of a xenotransplantation assay, Thanopoulou *et al.* ¹⁷ designed a study utilizing NOD/SCID mice that lacked $\beta 2$ microglobulin ($\beta 2m^{-/-}$) and expressed human IL-3, GM-CSF, and Steel factor (c-kit ligand). The authors reported that MDS cells from 9 of 11 patients successfully engrafted, and that 4 of the 5 samples that had a clonal cytogenetic marker, such as trisomy 8 or del 5q, engrafted the MDS clone. However, the engraftment level of the MDS clone was typically quite low, less than 1% of nucleated cells, and the mice did not develop clinical MDS. Kerbaux *et al.* ¹⁸ have recently reported the successful engraftment of human MDS clonal cells using the NOD/SCID- $\beta 2m^{-/-}$ model. However, the percent of MDS cells in the engrafted mice was small (0.14–4 %), and the mice did not develop clinical disease. Taken together, these studies demonstrate that human MDS cells have the capability to engraft in immunodeficient mice, but do not produce clinical disease. The challenge for future work is to improve the engraftment of the MDS clone, in sufficient numbers as to generate clinical disease in the engrafted mice.

Use of genetically engineered cells

In general, two approaches have been used to generate mouse hematopoietic cells that express (or, in the case of putative MDS tumor suppressors genes, do not express) genes thought to be relevant for the development of MDS. In the first approach, murine bone marrow nucleated cells (BMNC) are harvested and infected *in vitro* with a retroviral construct that expresses the gene of interest. The infected BMNC, including hematopoietic stem and progenitor cells which now express the gene of interest, are then transplanted into a lethally irradiated, syngeneic host mouse. The second general approach involves modification of the mouse germline, either by pro-nuclear injection of DNA into fertilized ova to generate transgenic mice, or by homologous recombination of mouse embryonic stem (ES) cells which can then be injected into blastocysts, leading to generation of chimeric mice. Experiments can be designed such that a gene of interest is deleted (“knocked out”), or so that a gene of interest is introduced (“knocked in”). In either case, this second general approach leads to mice that have genetically modified germline genomic DNA. More recently, a variety of approaches using conditional promoters (such as a tetracycline inducible promoter) or tissue specific transgenes have given investigators the opportunity to control expression in both a temporal and spatial fashion.

Pten/Ship

Because of the influence that Ship (src homology 2- containing 5' phosphoinositol phosphatase) and Pten (phosphatase and tensin homolog) have on the regulation of second messenger PIP3 and the fact that Ship-deficient mice develop a lethal myeloproliferative condition ^{19,20}, *Pten+/- Ship-/-* mice were developed ²¹. While hypothesized that these mice would develop leukemia, instead they displayed features consistent with aberrant

hematopoiesis. *Pten*^{+/-} *Ship*^{-/-} mice survived only about 5 weeks and during that time, anemia, leukocytosis, and thrombocytopenia were observed. There was no evidence of leukemia, however, extramedullary hematopoiesis in the liver was found. Although increased numbers of neutrophils were present in the peripheral blood, decreases in myeloid progenitors were revealed following methocellulose culture of the bone marrow. Bone marrow from *Pten*^{+/-} *Ship*^{-/-}²² mice failed to reconstitute the peripheral blood of irradiated wild type mice in a transplantation assay.

***Evi1* overexpression**

Transgenic *Evi1* mice were developed by Louz et al.,²³ by utilizing the *Sca-1* promoter for expression in hematopoietic stem cells. The expression of *Evi1* in the hematopoietic tissues of these mice resulted in a sex-specific response in one of the transgenic lines whereby females remained healthy during an 18-month period in contrast to their male littermates who manifested reduced CFU-E colony formation. These male mice became severely ill due to impaired erythropoiesis and died within 7 to 11 weeks. Although these mice did not develop MDS or leukemia, the potential for *Evi1* to collaborate in tumor formation was demonstrated by the ability of retrovirally-infected transgenic *Evi1* mice to develop myeloid leukemia more quickly than infected wild type mice.

However, expression of *Evi1* in a bone marrow transduction/transplantation model resulted in a progressive pancytopenia that was inevitably fatal by 12 months²⁴. Upon closer examination, necropsy revealed splenomegaly in *Evi1*-positive mice with an increase in erythroid precursors, iron deposition, and activated caspase-3 compared with spleens from control animals. Similar to clinical MDS, the bone marrow displayed hyperplasia of erythroid cells and megakaryocytes. In contrast to MDS in humans, the *Evi1*-induced MDS did not transform to leukemia in this study. Prior to the onset of overt disease, *Evi1*-positive mice at 4 months had low levels of *EpoR* and *c-Mpl* in bone marrow cells, which correlates with the defect in erythropoiesis and platelet generation observed in moribund mice months later. Follow-up studies observed that binding of *Evi1* to Gata1 resulted in impaired expression of the *EpoR*²⁵ while *Evi1* association with PU.1 altered myelopoiesis²⁶.

***Npm1* deletion**

Npm1 was of interest for study due to its frequently detected mutation in AML (35%), location on chromosome 5q, and ability to regulate tumor suppressor genes²⁷. One allele of *Npm1* was inactivated in mouse ES cells by homologous recombination that produced a deletion of exons 2 through 7. After generation of chimeric mice and germline transmission, the investigators noted that *Npm1*^{-/-} (*Npm1* “knockout”) mice were not viable. The *Npm1*^{-/-} mice died between embryonic day E11.5 and E16.5 due to severe anemia and aberrant organogenesis. *Npm1*^{+/-} mice were viable and demonstrated some features that were consistent with MDS²⁷. The majority of mice aged 6–18 months presented at least one blood abnormality commonly associated with MDS. *Npm1*^{+/-} mice often demonstrated an elevated MCV and had a wider range of platelet counts, both increased and decreased, than that of wild type mice, however, the hemoglobin levels did not differ between groups. Approximately two-thirds of the *Npm1*^{+/-} mice had morphologic evidence of erythroid or megakaryocytic dysplasia, including binucleated erythroblasts and hypolobated megakaryocytes. In a follow-up study, prolonged (up to 24 months) monitoring of *Npm1*^{+/-} mice revealed an increased incidence of malignancy compared to wild type mice, including lung and liver tumors, myeloproliferative neoplasms (MPNs), AML, B-cell lymphoma, and T-cell lymphoma²⁸.

***Dido1* deficiency**

Dido1 is a gene that was initially identified in a screen for genes whose expression was up-regulated during the initial stages of apoptosis. Homozygous deletion of *Dido1* in mice led to

a hematopoietic disease manifested by a variable degree of mild anemia and a variable degree of granulocytosis, in combination with decreased hematopoietic colony formation *in vitro*²². Dysplastic cells in the spleen and bone marrow were reported, but not characterized in detail, and progression to acute leukemia was not noted. The disease was thought to be consistent with a diagnosis of MDS/MPD and it was transplantable to recipient mice, indicating that it was cell-autonomous.

NUP98-HOXD13

Over 20²⁹ chromosomal translocations involving the *NUP98* gene associated with hematopoietic malignancy have been identified (for a review, see 30-31). Pineault *et al.*³² described a *NUP98-HOXD13* mouse model in which primary murine bone marrow cells were transduced with a retrovirus carrying a *NUP98-HOXD13* fusion gene, which was initially identified in a patient with MDS³³. These cells were subsequently transplanted into irradiated recipient mice. Significant decreases in WBCs were observed in mice expressing the *NUP98-HOXD13* fusion, which was further characterized by a reduction in B220+ and CD4+/CD8+ lymphocytes. In contrast, significant increases in myeloid bone marrow cells (Gr1+/Mac1+) were identified. Results from colony-forming unit spleen (CFU-S) assays with *NUP98-HOXD13*-transduced BM cells indicated a decrease in erythroid precursors (identified by the Ter119 cell surface marker), an increase in myeloid Gr1+ cells, as well as an overall increase in the number of CFU-S colonies at day 12. Although some of these mice developed a myeloproliferative disease as early as 4 weeks following transplantation, they did not transition to acute myeloid leukemia except when mice were engineered to simultaneously express *Meis1*.

In comparison, *NUP98-HOXD13* (*NHD13*) transgenic mice that utilized *Vav 1* regulatory elements to direct expression of the *NHD13* transgene in hematopoietic tissues developed anemia, neutropenia, and lymphopenia at 4–7 months⁵. These mice also had a variable degree of thrombocytopenia and macrocytosis, however, these differences were not statistically significant. Despite the peripheral blood cytopenias, these mice had hypercellular or normocellular bone marrows, indicating ineffective hematopoiesis. Morphologically, the *NHD13* mice showed dysplasia in the form of binucleate erythroblasts, megaloblastosis, pseudo-Pelger-Huet cells, and micromegakaryocytes. Similar to the progression pattern observed in patients with MDS, approximately half of the *NHD13* mice with MDS developed an acute leukemia, typically at 10–14 months of age. A variety of leukemic phenotypes were identified, most commonly AML. However, some animals developed pre-T lymphoblastic leukemia/lymphoma (pre-T LBL) which is rarely seen in secondary leukemia that evolves from patients with MDS. Although the development of pre-T LBL was somewhat surprising, it was not completely unexpected given that *NUP98* fusion genes have been associated with both T cell and myeloid malignancies (as discussed in 34). Of note, although the initial studies of the *NHD13* fusion used FVB/N mice, the entire study was subsequently repeated with C57Bl/6 mice. The results were remarkably similar in terms of peripheral blood cytopenias, dysplastic morphology, and progression to acute leukemia, demonstrating that the effect of the transgene was not mouse strain-dependent⁵. There are both similarities and difference between the transgenic and retroviral transduction/transplantation models. Both lead to anemia, lymphopenia, and neutropenia initially, however, the retrovirally transduced model quickly transforms to a myeloproliferative disease, whereas the transgenic mice transform more slowly, usually to an AML. The differences between the transgenic and retroviral transduction models could be due to differences in mouse strain, *ex vivo* manipulation of cells, effects from bone marrow reconstitution, or differential susceptibility of the bone marrow stem and progenitor cells by the *NUP98-HOXD13* retrovirus.

SALL4 overexpression

SALL4B is a zinc finger transcription factor expressed early during embryonic development, and is involved in the transcriptional regulation of *Oct4* and *Nanog*³⁵. *SALL4B* was constitutively expressed in mouse tissues under the control of the CMV promoter³⁶. These mice developed some classic features of MDS within a few months of life, including peripheral blood leukopenia and dysplasia, as well as a mild anemia. Bone marrow from these mice was hypercellular and showed an increased number of Mac1+/Gr1+ cells, as well as increased apoptosis when compared to wild type counterparts. Half of the transgenic mice transformed to acute myeloid leukemia by an average of 14.5 months of age and the disease was transplantable. In addition to the hematopoietic abnormalities, the majority of the transgenic mice were also diagnosed with polycystic kidneys and a minority was identified as having cerebral spinal fluid accumulation in the brain that affected motor function.

BCL-2 and NRAS

In an effort to study the process of MDS transformation to AML, overexpression of an inducible *BCL-2* transgene along with simultaneous expression of an *NRAS* mutant (NrasD12) were selected for examination in a mouse model³⁷. In collaboration with the increased proliferative effects attributed to mutant *NRAS*, it was predicted that a complementary mutation, induced by overexpression of anti-apoptotic *BCL-2*, would induce MDS or AML. The authors found that mice that expressed *BCL-2* and mutant *NRAS* developed a mild leukocytosis and thrombocytopenia, without anemia. The mice also displayed, hepatosplenomegaly, dysplastic neutrophils and increased bone marrow blasts (mean of 15%). Serial transplantations of the lin^{neg} but not the lin^{pos} cells from mice with MDS into immunodeficient mice were successful.

RUNX1 mutations

Watanabe-Okochi *et al.*³⁸ infected mouse bone marrow with retroviral constructs that encoded one of two different *RUNX1* (also known as *AML1*) gene mutations that were initially identified in patients with MDS or AML. The D171N mutation is a missense mutation involving the runt homology domain, and the AML1-S291fsX300 mutation is a frameshift mutation that results in truncation of the C-terminal region of the AML1 protein. The majority of mice that expressed the D171N *RUNX1* mutation developed MDS with an increased RBC mean corpuscular volume (MCV), dysplasia, anemia, leukopenia and progression to AML³⁸ (Table 2). Of note, a fraction of the *RUNX1*-D171N mice had integrations of the retroviral vector used to express the *RUNX1* mutant at the *Evi1* locus, and transplantation of bone marrow cells co-infected with *RUNX1*-D171N and *Evi1* reduced the latency of the disease to 3 to 5 months. This study provides support for the cooperation of *RUNX1* mutants and *Evi1* for inducing MDS with progression to AML.

Arid4a deficiency

ARID4A and ARID4B (also known as RBBP1 and RBBP1L1) are proteins which contain an AT-rich interaction domain (ARID). ARID4A, but not ARID4B, interacts with RB and recruits histone deacetylases (HDAC) to E2F-dependent promoters³⁹. Arid4a deficient (*Arid4a*^{-/-}) mice were identified by a gene trap mutagenesis screen⁴⁰. Young (2–5 month old) *Arid4a*^{-/-} mice developed lymphopenia, neutropenia, thrombocytopenia, and a mild anemia, with a normocellular bone marrow⁴¹. The peripheral blood cytopenias described above became progressively more severe, with a marked increase in monocytes, as the mice aged. These changes were accompanied by bone marrow fibrosis with increased reticulin fibers and hepatosplenomegaly secondary to a compensatory extramedullary hematopoiesis. These features are reminiscent of a CMML that has evolved from a MDS/MPD. In 12% of the mice, the disease transformed to a frank AML, with high peripheral blood WBC, at ages between 12 and 22 months. BM from the *Arid4a*^{-/-} mice had decreased levels of several *Hoxb* cluster

genes(*Hoxb3,5,6,8*) that are normally expressed in BM, but levels of *Hoxa7*, *Hoxa9*, and *Meis1*, which are upregulated in some MDS cases in mice and patients (see above), were normal⁴¹. Although mice that had deleted both *Arid4a* and *Arid4b* were not viable (*Arid4a*^{-/-} *Arid4b*^{-/-}), *Arid4a*^{-/-} *Arid4b*^{+/-} developed AML with a higher frequency (83%) at an early age (7–15 months).

DNA polymerase gamma (*Polg*) mutations

Although controversial due to the inexorable accumulation of mitochondrial (mt) DNA mutations with age^{42,43}, some studies have suggested that MDS patients have an increased frequency of mt DNA mutations^{44–46}. DNA polymerase gamma (*Polg*), a protein which is encoded by the nuclear genome, is responsible for replication of mitochondrial (mt) DNA. *Polg* has two enzymatic activities: a C-terminal polymerase and an N-terminal “proofreading” exonuclease. Mice with targeted instability of the mitochondrial genome were produced by selective elimination of the N-terminal proofreading activity through gene-targeted creation of a missense mutation (*Polg* D257A); the mutant allele is designated *Polg*^{A47}. Homozygous mutant mice (designated *Polg*^{A/A}) show an increase in the frequency of mt DNA mutations and exhibit features of premature aging, including hair loss, thymic involution, testicular atrophy, and loss of intestinal crypts. In addition, the mice showed a progressive, fatal severe megaloblastic anemia and impaired lymphopoiesis, but there was no evidence for transformation to acute leukemia⁴⁸. Of note given the loss of intestinal crypts in the *Polg*^{A/A} mice, the megaloblastic anemia was demonstrated to be due to mitochondrial defects in the hematopoietic compartment, as opposed to malabsorption, by transplantation of *Polg*^{A/A} cells in wild-type recipient mice.

Clinical implications of modeling MDS in mice

Mouse models are developed in order to study, in detail, questions that cannot easily (or are impossible to) be addressed in the clinic. A useful animal model for MDS would accurately represent genetic aberrations in patients, provide similar micro-environmental conditions for reproducing hematopoietic phenotypes (*e.g.* impaired differentiation and increased apoptosis), recapitulate patient symptoms and responses to existing treatment regimens, as well as progress to AML with similar kinetics and features as seen in the clinic. While a model that satisfies all of these criteria may not exist, cancer biologists have several different models to choose from, and can select an available animal model, being cognizant of its caveats. Once an MDS model is developed and validated, it can be utilized to advance the understanding of the molecular biology of the disease and provide a platform for cultivation of new therapeutic approaches. This section will be focused on mice with *NUP98-HOX* fusions, as there have been a number of follow-up studies^{34,49–54} performed with these mice beyond the initial description, providing examples for the types of additional studies that are possible with these animal models.

Although rare, many of the *NUP98* translocations, including *NUP98-TOP1*, *NUP98-DDX10*, and *NUP98-HOX* fusions, have been seen in patients with MDS, especially younger patients^{33,55–59}. Furthermore, the *NUP98-HOXA9*, and *NUP98-HOXD13* translocations both lead to overexpression of *HOXA9* in mice; marked (10 to 1000-fold greater than normal bone marrow or CD34+ cells) overexpression of *HOXA9* is a common finding in patients with AML⁶⁰ and MDS⁶¹. *NHD13* mice develop a variety of leukemias including myeloid, erythroid, megakaryocytic, and undifferentiated, however, although unexpected, about 15–25% of *NHD13* mice developed pre-T or pre-B LBLs^{53,4}. This is in contrast to reports of human MDS transformation to lymphoid leukemias, which are rare^{62–65}. Despite this difference between MDS patients and mouse models of the disease, 60% of *NHD13* mice developed acute leukemias and 20% died because of severe MDS, statistics that are similar with patient progression³⁴.

Additional studies based on the *NHD13* translocation has sought to describe the implications of this mutation and to understand its mechanistic role in MDS. The *NHD13* protein can inhibit maturation of the K562 cell line, suggesting that *NHD13* expression plays a role in hematopoietic precursors by impairing their differentiation⁵. In order to study the impaired differentiation attributed to the *NHD13* protein, colony-forming cell assays were performed with bone marrow from the *NHD13* transgenic mice. Although the number of colonies formed by plating whole bone marrow from wild type and *NHD13* mice were similar, ten-fold fewer total colonies were observed from the *NHD13* lin^{neg} bone marrow population (which contains the stem and progenitor cells) than from the wild-type lin^{neg} bone marrow cells⁶⁶. Lin^{neg} cells from *NHD13* mouse bone marrow also showed dramatic increases in apoptosis following treatment with hematopoietic cytokines that promote survival and differentiation of wild-type murine hematopoietic stem and progenitor cells. Moreover, the majority of the surviving *NHD13* cells were undifferentiated Lin^{neg} cells. Taken together, these findings indicate that the *NHD13* fusion impairs differentiation of hematopoietic stem and progenitor cells and predisposes these cells to apoptotic death; these findings are reminiscent of the maturation arrest and increased apoptosis seen in patients with MDS.

Subsequent studies in the lymphoid system of *NHD13* mice revealed impaired B-lymphocyte differentiation at the pro-B to pre-B transition and impaired differentiation of T-lymphocytes during the transition from double negative (DN) DN2 to DN3 thymocytes⁴⁹. *Hoxa7*, *Hoxa9* and *Hoxa10* were overexpressed in the thymus of *NHD13* mice compared to that of wild type mice, an association that has previously been observed with impaired thymocyte differentiation.

Additional studies have identified a long-term repopulating MDS-initiating cell (MIC) by demonstration of successful engraftment of Lin^{neg} *NHD13*-expressing bone marrow cells into primary and secondary lethally-irradiated wild type recipients. Importantly, mice that received the *NHD13* bone marrow developed signs and symptoms of MDS, including peripheral blood cytopenias due to ineffective hematopoiesis, morphologic dysplasia, and transformation to acute leukemia⁵¹. These studies also demonstrated that, in a competitive repopulation assay, the *NHD13* bone marrow cells were able to out-compete normal wild-type bone marrow cells, even when a ten-fold excess of wild-type bone marrow was transplanted. Finally, limiting dilution experiments identified the frequency of the MDS-initiating cell to be similar to the frequency of hematopoietic stem cells from wild type mice (~1/20,000).

In addition to elucidating the role of *NHD13* in MDS, its contribution to the development of leukemia is also of interest. In the context of the recently developed 2-class mutation hypothesis for AML⁶⁷, the *NUP98-HOX* fusions can be classified as Type II, or differentiation-impairing, mutations⁵²⁻⁶⁸. Infection of primary bone marrow cells by replication-competent retroviral particles predisposes mice to develop leukemia through a process known as retroviral insertional mutagenesis (see⁶⁹ for a review). This happens due to integration of the retrovirus, with the retroviral LTR serving as a powerful promoter/enhancer, near to cellular proto-oncogenes. Activation of two or more collaborative proto-oncogenes leads to leukemia or lymphoma in the infected mice. When *NHD13* mice were infected with the MOL4070LTR retrovirus, the time from MDS to leukemic transformation was shortened by six months, providing further support that a second event is needed in collaboration with the *NHD13* fusion for progression to leukemia⁵³. Potential collaborators with *NHD13* identified by retroviral insertional mutagenesis include: *Meis1*, *Mn1*, *Gata2*, *miR29a* and *miR29b1*⁵³.

In an effort to identify spontaneous mutations that might collaborate with *NHD13* as the MDS converts to an AML, genes frequently mutated in human AML were re-sequenced in leukemic tissue from *NHD13* mice that had developed AML. In *NHD13* mice with AML, 32% of mice assessed were found to have a *Nras* or *Kras* mutation of codon 12⁵⁴. Interestingly, no codon

13 or 61 mutations were found, although a codon 61 *Nras* mutation was identified in a *NHD13* mouse with a pre-T LBL. One mouse was found to have a mutation in *Cbl*, specifically an exon 8 deletion. These results, along with the lack of mutations observed in *Npm1* and *Runx1* (both classified as Type II, or differentiation-blocking mutations) are again consistent with *NHD13* being a Type II mutation. Given that *KIT* mutations are most commonly found in patients with core-binding factor (CBF) translocations, it may not have been surprising that *Kit* mutations were not identified in the *NHD13* AMLs, as the *NHD13* fusion does not seem to affect the CBF pathways. However, it was curious that no *Flt3* mutations were identified, as *Flt3* mutations are commonly associated with lesions (such as fusions of the *MLL* gene) that upregulate *HOXA* cluster genes⁵⁴.

In addition to verifying that mutations (such as *Runx1* point mutations or *NUP-HOX* gene fusions) found in MDS patients are important in the disease process, mouse models provide a platform that can be used for pre-clinical testing. In addition to testing small molecules, singly or in combination, these animal models can be used to study the mechanism(s) by which allogeneic hematopoietic stem cell transplant (HSCT) is curative for patients with MDS. HSCT is the only known definitive treatment for MDS patients, and is thought to be effective by a combination of myeloablative radio-chemotherapy and adoptive immunotherapy leading to a graft versus tumor effect. Inbred strains of mice with complete or partial MHC mismatches afford the ability to test the relative contributions of these two effects. In addition, these animal models may also be useful for examining possible autoimmune contributions to MDS initiation as proposed when immunosuppressive therapy resulted in positive responses in certain patient subsets^{70–72}.

Despite the challenges encountered in the long term growth and proliferation of primary MDS cells *in vitro* and *in vivo*, modeling the disease in mice has recently met with some success. Additional studies will be needed to more fully characterize the genetic lesions that lead to MDS, as well as which complementary mutations lead to leukemic transformation of the MDS clone. This information, along with insight into the fundamental basis for the impaired differentiation and apoptosis associated with MDS may be extracted from many of the models discussed here. It is our hope that studies utilizing the recently described animal models discussed above will be useful in developing new therapeutic approaches for patients with MDS in the near future.

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Table 1

Criteria for the diagnosis of myeloid dysplasias in mice.

1	At least one of the following findings in peripheral blood:
A	Neutropenia
B	Thrombocytopenia (in the absence of leukocytosis or erythrocytosis)
C	Anemia (in the absence of leukocytosis or thrombocytosis)
2	Detection of a maturation defect in non-lymphoid hematopoietic cells manifested by at least one of the following:
A	Dysgranulopoiesis, dyserythropoiesis, or dysplastic megakaryocytes, with or without increased non-lymphoid immature forms or blasts
B	At least 20% non-lymphoid immature forms or blasts in the bone marrow or spleen
3	Disorder does not meet the criteria for a non-lymphoid leukemia.
4	If the disorder complies with:
2A	then the subclassification is a <i>myelodysplastic syndrome</i>
2B	then the subclassification is <i>cytopenia with increased blasts</i>

Data from Kogan et al., Blood 2002; 100:238–45

Table 2

Summary of genetically engineered mouse models for MDS

Gene(s)	Technique	Findings	Reference
<i>Pten/Ship</i>	<i>Pten</i> haploinsufficient <i>Ship</i> knockout	Leukocytosis, neutrophilia, anemia, thrombocytopenia, hepatosplenomegaly, extra-medullary hematopoiesis. Lethal by 5 weeks of age, no leukemia.	21
<i>Evi1</i>	Retroviral transduction	Anemia, thrombocytopenia, variable leukopenia, hypercellular BM, BM dysplasia, uniformly fatal (14 mos), no leukemia.	24
<i>Npm1</i>	<i>Npm1</i> haploinsufficient	No anemia, variable thrombocytosis or thrombocytopenia, BM dysplasia and hypercellularity. Lung, liver tumors, AML, lymphoma at advanced age.	27,28
<i>Dido</i>	Knockout	Variable anemia, variable leukocytosis, variable splenomegaly, BM dysplasia. Survival 55% at 26 mos, no leukemia.	22
<i>NUP98-HOXD13</i>	Transgenic (<i>Vav</i> promoter)	Anemia, macrocytosis, leukopenia, variable thrombocytopenia, hyper/normo-cellular BM, dysplasia. Survival 5% at 14 months, 60% leukemic transformation (AML and ALL).	5,49–51
<i>SALLAB</i>	Transgenic (CMV promoter)	Mild anemia, mild neutropenia, variable thrombocytosis, BM dysplasia and hypercellularity. Survival 12% at 24 months, 50% leukemic transformation (AML).	36
<i>BCL2/NRASD12</i>	Transgenic (tTA, MRP8 promoters)	No anemia, mild leukocytosis, mild thrombocytopenia, BM dysplasia, hepato-splenomegaly, survival/leukemia not reported.	37
<i>RUNX1</i>	Retroviral transduction (S291fs)	Anemia, macrocytosis, leukopenia, thrombocytopenia, hyper/normocellular BM, dysplasia. Survival 10% at 14 months, 60% leukemic transformation.	38
<i>Arid4a</i>	Knockout	Mild anemia, leucopenia, lymphopenia, thrombocytopenia, hyper/normocellular BM with fibrosis. 12% AML at 12–22 months.	41
<i>Polg</i>	Knock-in of mutant (<i>Polg^{A/A}</i>)	Anemia, lymphopenia, BM dysplasia, mitochondrial dysfunction. Lethal by 16 months due to severe megaloblastic anemia.	48