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NUP98-HOX translocations lead to myelodysplastic syndrome in mice and men

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Abstract

The myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis, peripheral blood cytopenias, dysplasia and a propensity for transformation to acute myeloid leukemia (AML). A wide spectrum of genetic aberrations has been associated with MDS, including chromosomal translocations involving the *NUP98* gene, most commonly leading to fusions of *NUP98* with *abd-b* group *HOX* genes, including *HOXD13*. We used *vav* regulatory elements to direct expression of a *NUP98-HOXD13 (NHD13)* fusion gene in hematopoietic tissues. *NHD13* transgenic mice faithfully recapitulate all of the key features of MDS, including peripheral blood cytopenias, bone marrow dysplasia and apoptosis, and transformation to acute leukemia. The MDS that develops in *NHD13* transgenic mice is highly lethal; within 14 months, 90% of the mice died of either leukemic transformation or severe anemia and leukopenia due to progressive MDS. These mice provide a pre-clinical model that can be used for the evaluation of MDS therapy and biology.

Keywords

NUP98; HOXD13; Myelodysplastic syndrome; Leukemia; Transgenic mouse

Introduction

Myelodysplastic syndrome (MDS) is a heterogenous group of diseases characterized by dysplasia, ineffective hematopoiesis, and peripheral blood cytopenias. Clinically, MDS typically has one of three outcomes. Patients may die due to complications of pancytopenia, the disease can transform to an acute leukemia, or patients may survive for an extended period of time with the disease (1). The crude incidence of MDS has been estimated at 3.5–12.6 per 100,000 per year, and there is a suggestion that this incidence may be increasing (2). A large number of chromosomal abnormalities, including deletions, amplifications, inversions, and translocations have been identified in the malignant cells of patients with MDS (1,3,4).

A new class of chromosomal aberration associated with MDS

The most common recurrent genetic abnormalities in patients with MDS are deletions of chromosomes 5q, 7q, and 20q (1,3,4). Despite decades of intensive study, and numerous

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promising leads, the critical gene(s) located in these regions that are responsible for MDS have not conclusively been identified. Recently, a number of chromosomal translocations involving the *NUP98* gene, located on chromosome 11p15.5, have been identified (5) in patients with hematologic malignancy. *NUP98* translocations have now been recognized in a wide array of hematologic maliganancies, including MDS, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), and precursor-T lymphoblastic lymphoma/leukemia (pre-T LBL) (5). With the use of increasingly sophisticated cytogenetic techniques, at least 20 different partner genes for *NUP98* have now been identified. Remarkably, half of the partner genes encode homeodomain proteins, primarily those belonging to the *abd-b* group of *HOX* genes (Table 1). As indicated in table 1, the *NUP98-HOX* gene fusions are universally associated with myeloid malignancies, whereas the *NUP98* fusions involving non-homeodomain genes are associated with a wider spectrum of hematologic malignancies.

The *NUP98* gene encodes a 98 kD component of the nuclear pore complex (NPC) that mediates nucleo-cytoplasmic transport of RNA and protein (6,7). NUP98 contains N-terminal phenylalanine-glycine (FG) repeats, which are commonly found in NPC proteins. The *NUP98* gene fusions associated with hematologic malignancy invariably encode fusion proteins that fuse the amino-terminal portion of NUP98, containing the FG repeats, with the carboxy-terminal portion of the partner gene (5). In the case of NUP98-HOX fusions, the DNA-binding homeodomain is contained in the NUP98-HOX fusion protein.

Although the molecular mechanism(s) that generate chromosomal translocations involving *NUP98* remain poorly understood, it is important to note that many of the *NUP98* translocations have been recognized in patients with therapy-related AML or MDS (t-AML or t-MDS). Intriguingly, analysis of breakpoints from t-MDS patients with *NUP98-TOP1* fusions revealed an almost perfect reciprocal chromosomal translocation, consistent with a topoisomerase II “subunit exchange” model for the translocation (8).

A mouse model for MDS

Our lab has used two approaches in an attempt to generate an *in vivo* model for MDS using an *NHD13* fusion gene. In our first approach, we used homologous recombination to “Knock-In” a *HOXD13* cassette at mouse *Nup98* exon 12 (9). Although we were able to successfully target the *Nup98* locus and produce a *NUP98-HOXD13* (hereafter *NHD13*) mRNA and protein, we were unable to generate chimeric mice, as there was no contribution from the ES cells to adult mouse tissues. The *NHD13* ES cells grew as rapidly as the parental cells *in vitro*, and we were able to detect contributions from the ES cells to chimeric mice up to embryonic day 14.5, consistent with a hypothesis that the *NHD13* ES cells were impaired in their ability to differentiate and contribute to adult tissues. We next attempted to differentiate the *NHD13* ES cells *in vitro*, using a cytokine cocktail including IL3, IL6, SCF, and Epo. In this experiment, although the parental ES cells differentiated to CFU-GM, CFU-GEMM, and CFU-E colonies, the *NHD13* cells primarily formed dense clusters of cells reminiscent of blast colonies (BL-CFC) (10). Morphologically, these cells had an appearance consistent with undifferentiated blast cells, displaying a high nuclear/cytoplasmic ratio, uncondensed chromatin, and prominent nucleoli (9). These findings again suggest that the *NHD13* ES cells are impaired in their ability to differentiate.

In a complementary approach, we generated transgenic mice on an FVB/N background that express an *NHD13* fusion gene under the control of *vav* regulatory elements (11), leading to expression of the *NHD13* fusion gene in all hematopoietic tissues (12). These mice did develop MDS, with leukopenia, neutropenia, and anemia (Table 2) despite a hypercellular or normocellular bone marrow (Figure 1). The disease was highly lethal, as over 90% of the *NHD13* transgenic mice died by 14 months of age. Similar to human MDS, 20% of the mice

died of severe pancytopenia without evidence of transformation to acute leukemia, 20% of the mice died of unknown causes, and 60% of the mice progressed to acute leukemia.

The leukemic subtype was assessed by FACS, immunohistochemistry, gene expression profiling, and T-cell receptor gene rearrangements. Surprisingly, half of the leukemias we identified in our initial series were pre-T lymphoblastic leukemia/lymphoma (pre-T LBL), with the remainder being non-lymphoid, most commonly myeloid, leukemias. The finding of pre-T LBL in *NHD13* mice was unanticipated, since human MDS only rarely transforms into a lymphoid malignancy (13,14), and since *NHD13* fusions have not been identified in human patients with T-cell malignancies. However, it should be noted that other *NUP98* fusion genes, such as *NUP98-RAP1GDS1* and *NUP98-ADD3*, have been associated with pre-T LBL (15, 16), leading to the suggestion that expression of *NUP98* fusion genes can result in T-cell malignancies as well as myeloid malignancies. Furthermore, one of the *NHD13* mice simultaneously had both an erythroid leukemia and a pre-T LBL (Figure 2), raising the possibility that the erythroid leukemia had evolved from a pre-existing MDS, and the pre-T LBL had originated in the thymus from thymic precursors independent of the MDS clone, and infiltrated the lung (but not liver or spleen). Therefore, we favor the hypothesis that the pre-T LBL in the *NHD13* mice arises as an independent malignancy, as opposed to arising from an MDS clone. In either case, these findings indicate that the *NHD13* transgene is oncogenic in T-lymphoid precursors as well as myeloid and erythroid cells.

Since the above results were based on offspring from a single founder mouse, we generated additional transgenic lines to eliminate the possibility that the MDS phenotype was secondary to an insertional effect of the transgene; these additional lines were generated on a C57Bl6 background to determine whether the MDS phenotype was strain-specific. Similar to the findings seen on an FVB/N background, these mice showed peripheral blood cytopenias, dysplasia, and transformation to acute leukemia, with a similar high penetrance and similar age of leukemic transformation. In addition to erythroid, myeloid, and pre-T leukemias, some of these mice developed an undifferentiated leukemia that was negative for T-cell (CD3), B-cell (B220), myeloid (myeloperoxidase, MPO), monocyte (F4/80), and megakaryocytic markers (CD41). A single mouse developed a biphenotypic leukemia, characterized by thymic enlargement and hepatosplenomegaly, with infiltration of blasts that were positive for both CD3 and MPO.

Out of 51 transgene positive mice that were followed for at least 14 months, 7 were euthanized because the mice were sick or moribund, and showed signs of MDS, but had not transformed to acute leukemia. Of these 7 mice, three (#1145, 1899, 1196) had severe anemia (hgb < 5.0 g/dl), one (#2747) had a pulmonary hemorrhage, and one (#1903) had a retro-orbital infection, all signs consistent with severe pancytopenia.

The findings we describe here are distinct from results obtained with transduction of bone marrow mobilized with 5-fluorouracil and transduced with an *NHD13* retrovirus (17). In those experiments, *NHD13* both promoted growth and inhibited differentiation of hematopoietic progenitors *in vitro*; the inhibition of differentiation was characterized by a marked decrease in ter119+ erythroid cells in a colony forming unit-spleen (CFU-S) assay. Mice reconstituted with bone marrow that expressed the *NHD13* retrovirus initially displayed a diminished engraftment of cells that expressed NHD13, and leukopenia, principally due to lymphopenia. A minority of these mice developed anemia and leukocytosis, consistent with a myeloproliferative disease (MPD), whereas other mice from this cohort were markedly anemic but had normal WBC levels. Therefore, both the retroviral transduction and transgenic models lead to anemia and lymphopenia in mice, but the retroviral transduction model leads to MPD with increased neutrophil counts in a minority of mice, whereas the transgenic model leads to decreased neutrophil counts and frequent transformation to acute leukemia. The reasons for

the differences in these two models are not clear, but could be due to mouse strain differences, the nature of the cells targeted for retroviral infection, *in vitro* expansion of infected cells, activation of host genes located at the retroviral insertion sites, and/or relative levels of *NHD13* expression.

It has been suggested that mutations in at least two pathways, one leading to impaired differentiation, and one leading to increased proliferation and/or decreased apoptosis are required to produce AML (18). We strongly suspect that the NHD13 fusion protein exerts an oncogenic effect through impaired differentiation for several reasons. *NHD13* ES cells do not contribute to adult chimeric mice, and fail to differentiate *in vitro* (discussed above). In addition, simultaneous expression of a *NUP98-HOXA9* fusion and *BCR-ABL* fusion leads to a fulminant AML in a mouse transduction/transplantation model, whereas expression of *BCR-ABL* (a "proliferative class" mutation) alone leads to a non-fatal myeloproliferative disease (19). Furthermore, as opposed to cells transfected with an empty vector, K562 cells expressing an *NHD13* fusion fail to differentiate to megakaryocytes following treatment with phorbol esters (12). Finally, some human CML patients, who have a "proliferative" mutation (*BCR-ABL*), develop a *NUP98-HOX* translocation at the time of blast crisis and transformation to AML (20).

Outstanding questions and future directions

MDS is a heterogeneous group of diseases, that displays a wide spectrum of chromosomal abnormalities. Although rare, at least seven different *NUP98* translocations, including the *NUP98-HOXD13* translocation, have been recognized in patients with therapy-related MDS. *NHD13* mice recapitulate all of the key findings of human MDS, including blood cell dysplasia, peripheral blood cytopenias, ineffective hematopoiesis, and transformation to acute leukemia, and thus provide an excellent model for the human disease. Our future and ongoing studies are designed to address the following questions. First, we suspect that additional mutations, especially those in proliferative and/or apoptotic pathways, are required to convert the MDS to an acute leukemia. What are those mutations? We have begun experiments using retroviral insertional mutagenesis to identify collaborating genes; based on historical precedent, we anticipate that this approach will primarily identify activation or gain of function events. Complementary experiments that are more likely to identify loss of function events include screens using restriction landmark genome scanning (RLGS) (21) and array-based comparative genomic hybridization (aCGH) (22). We also have begun experiments designed to determine the utility of these mice as a pre-clinical model for drug development. We think that this type of model is particularly important for assessing a disease such as MDS, since available MDS cell lines typically have been established from MDS patients that have converted to AML, or have acquired additional chromosomal abnormalities, suggesting that these MDS cell lines are indistinguishable from AML cell lines (23). Finally, we are interested in determining if MDS, which can be viewed as a "pre-malignant" condition, at least in some cases, can be transplanted prior to conversion to AML.

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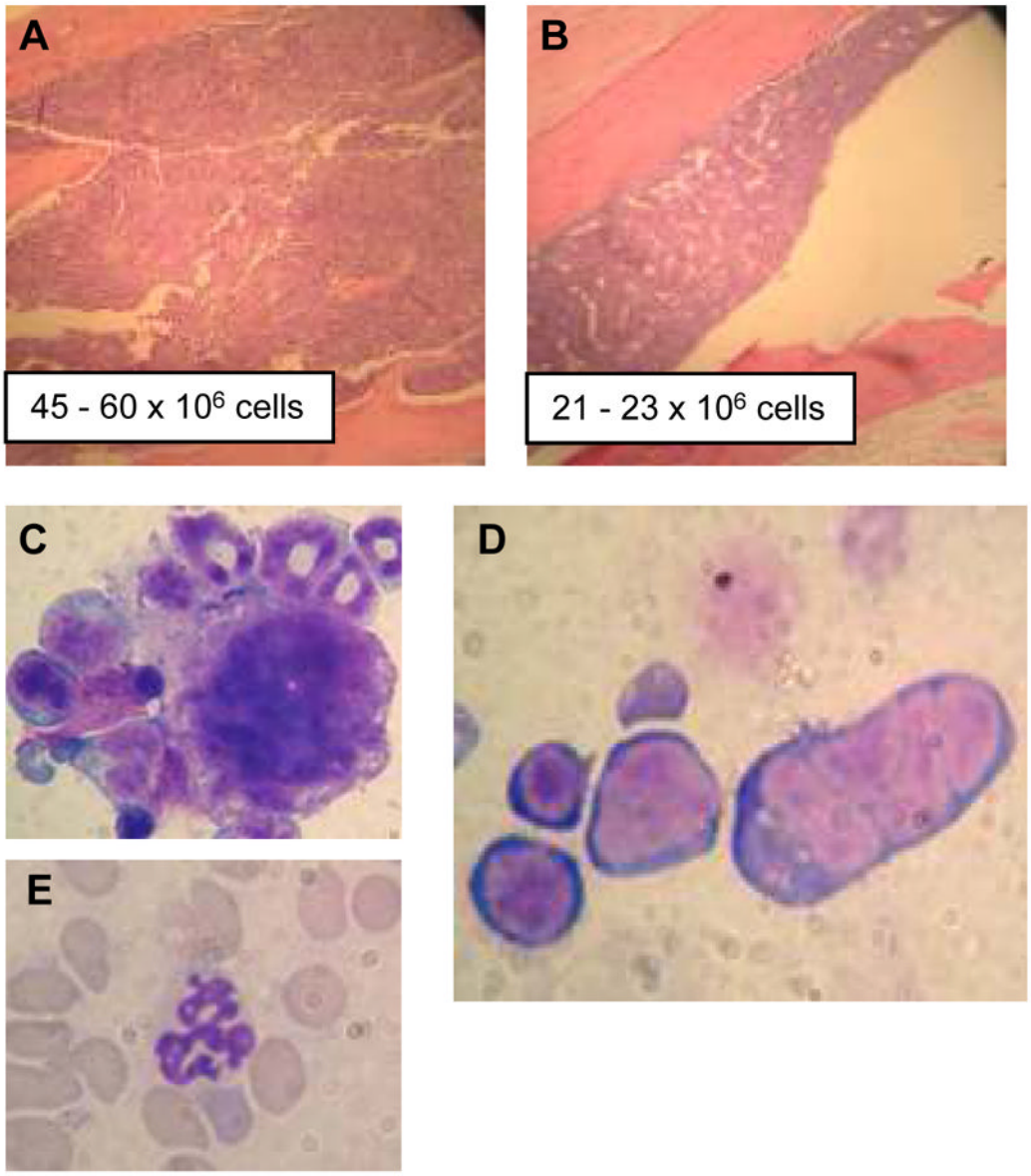

Figure 1. Ineffective hematopoiesis and dysplasia in NHD13 mice

A and B H &E stained bone marrow from NHD13 **(A)** and wild-type **(B)** mice; total cell number from 2 femurs are indicated. Note densely cellular bone marrow in **(A)** (original magnification × 100). Examples of a dysplastic micromegakaryocyte **(C)** and multinucleate erythroblast **(D)** and hypersegmented neutrophil **(E)** from NHD13 mouse are shown (original magnification × 1000).

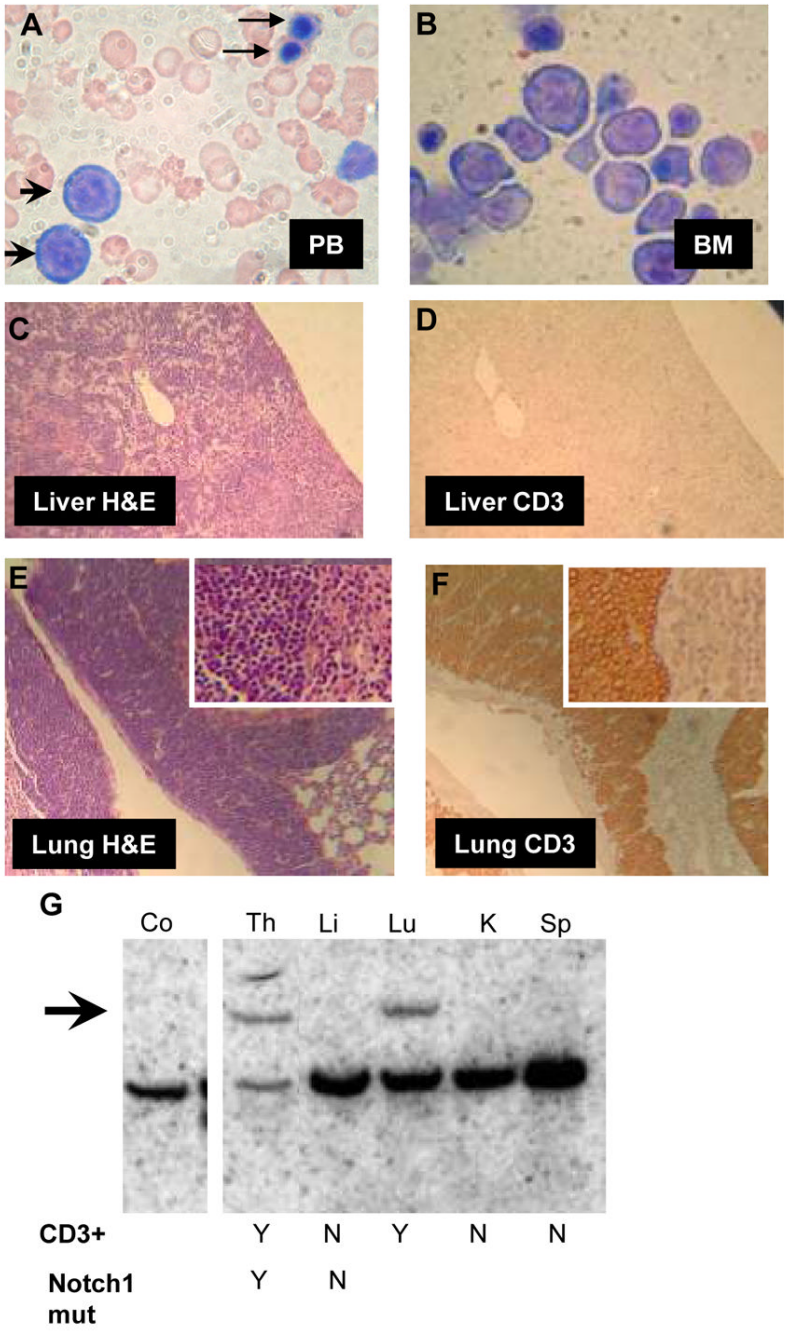

Figure 2. Concurrent erythroid and pre-T leukemia in NHD13 mouse 1018

A and B, peripheral blood **(A)** and bone marrow **(B)** demonstrating circulating erythroblasts (arrowheads), nucleated red blood cells (arrows), and replacement of bone marrow with erythroblasts (original magnification × 1000). **C and D**, section of liver infiltrated with leukemic blasts, stained with H&E **(C)** or anti-CD3 **(D)** (original magnification × 100). Note lack of CD3 staining in liver. **E and F**, section of lung infiltrated with leukemic blasts, stained with H&E **(E)** or anti-CD3 **(F)**. Note strong CD3 staining in lung (original magnification × 100; inset × 400). **(G)** Southern blot of leukemic tissue hybridized to a TCRB probe. Note germline band in all lanes, and a clonal rearranged band in the Th and Lu (arrow). CD3 staining

and presence of Notch1 mutation in infiltrated tissues is indicated as yes (Y) or no (N). Co, non-transgenic control; Th, thymus; Li, liver; Lu, lung; K, kidney; Sp, spleen.

Table 1

NUP98 translocations associated with hematologic malignancy

Translocation	Partner Gene	Homeodomain?	Disease
t(7;11)(p15;p15)	*HOXA9, 11, 13*	Yes	MDS, AML, CML
t(11;12)(p15:q13)	*HOXC11,13*	Yes	MDS, AML
t(2;11)(q31;p15)	*HOXD9, 11, 13*	Yes	MDS, AML, CML
t(1;11)(q23;p15)	*PMX1(PRRX1)*	Yes	MDS, AML
t(9;11)(q34;p15)	*PRRX2*	Yes	MDS, AML
t(4;11)(q21;p15)	*RAP1GDS1*	No	pre-T LBL
t(11;20)(p15;q11)	*TOP1*	No	MDS, AML
t(9;11)(p22;p15)	*LEDGF*	No	AML
t(5;11)(q35;p15)	*NSD1*	No	AML
t(8;11)(p11;p15)	*NSD3*	No	AML
t(10;11)(q25;p15)	*ADD3*	No	pre-T LBL
inv11(p15q22)	*DDX10*	No	MDS, AML
t(6;11)(q24;p15)	*C6orf80*	No	AMKL, pre-T LBL
t(3;11)(p24;p15)	*TOP2B*	No	AML
t(11;21;12)(p15;p13;p13)	*JAR1D1A*	No	AMKL

Table 2

CBC from *NHD13* transgenic mice aged 4–7 months

	WBC[a]	Neutrophil[a]	Lymph[a]	Hgb[b]	Plt[a]	BM blasts	BM erythroid dysplasia	BM myeloid dysplasia	BM mega dysplasia
Transgenic[c]	1.8 +/− 0.5	0.4 +/− 0.2	1.2 +/− 0.4	11.8+/− 2.1	958 +/− 569	14.1 ± 1.9%	12.8 ± 2.1%	6.0 ± 1.3%	rare
Control[c]	6.5 +/− 1.8	1.4 +/− 0.9	4.8 +/− 0.9	14.2 +/− 0.7	841 +/− 130	5.6 ± 1.8 %	1.5± 0.9%	1.8 ± 1.0%	rare
p value[d]	<0.001	<0.001	<0.001	<0.001	0.709	<0.01	<0.01	<0.05	

[a] White blood cell, neutrophil, lymphocyte, and platelet count expressed as 10^9/L.

[b] Hemoglobin in g/dL

[c] n=22 for transgenic CBC, n=7 for control CBC, n=5 for transgenic bone marrow, n=5 for control bone marrow

[d] *p* value by Mann-Whitney U-test.