NUP98-HOX Translocations Lead to Myelodysplastic Syndrome in Mice and Men

Christopher Slape, Ying Wei Lin, Helge Hartung, Zhenhua Zhang, Linda Wolff, Peter D. Aplan

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 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 preclinical model that can be used for the evaluation of MDS therapy and biology.

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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 promising leads, the critical genes 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 malignancies, including MDS, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), and precursor-T lymphoblastic lymphoma/leukemia (pre-T LBL) (3). 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 nonhomeodomain 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 mechanisms 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 laboratory 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, because there was no contribution from the embryonic stem (ES) cells to adult mouse tissues. The NHD13...
Table 1. NUP98 translocations associated with hematologic malignancy

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Partner Gene</th>
<th>Homeodomain?</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(7;11)(p15;p15)</td>
<td>HOXA9, 11, 13</td>
<td>Yes</td>
<td>MDS, AML, CML</td>
</tr>
<tr>
<td>t(11;12)(p15;q13)</td>
<td>HOXCl13,13</td>
<td>Yes</td>
<td>MDS, AML</td>
</tr>
<tr>
<td>t(2;11)(q31;p15)</td>
<td>HOXD9, 11, 13</td>
<td>Yes</td>
<td>MDS, AML, CML</td>
</tr>
<tr>
<td>t(11;11)(q23;p15)</td>
<td>PMX1(PRRX1)</td>
<td>Yes</td>
<td>MDS, AML</td>
</tr>
<tr>
<td>t(9;11)(q34;p15)</td>
<td>PRRX2</td>
<td>Yes</td>
<td>MDS, AML</td>
</tr>
<tr>
<td>t(4;11)(q21;p15)</td>
<td>RAP1GDS1</td>
<td>No</td>
<td>pre-T LBL</td>
</tr>
<tr>
<td>t(11;20)(p15;q11)</td>
<td>TOP1</td>
<td>No</td>
<td>MDS, AML</td>
</tr>
<tr>
<td>t(9;11)(p22;p15)</td>
<td>LEDGF</td>
<td>No</td>
<td>AML</td>
</tr>
<tr>
<td>t(5;11)(q35;p15)</td>
<td>NSD1</td>
<td>No</td>
<td>AML</td>
</tr>
<tr>
<td>t(8;11)(p11,p15)</td>
<td>NSD3</td>
<td>No</td>
<td>AML</td>
</tr>
<tr>
<td>t(10;11)(q25;p15)</td>
<td>ADD3</td>
<td>No</td>
<td>pre-T LBL</td>
</tr>
<tr>
<td>inv(11)(p15q22)</td>
<td>DDX10</td>
<td>No</td>
<td>MDS, AML</td>
</tr>
<tr>
<td>t(6;11)(q24,p15)</td>
<td>C6orf80</td>
<td>No</td>
<td>AMKL, pre-T LBL</td>
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<tr>
<td>t(3;11)(p24;p15)</td>
<td>TOP2B</td>
<td>No</td>
<td>AML</td>
</tr>
<tr>
<td>t(11;12;12)(p15,p13;p13)</td>
<td>JAR1D1A</td>
<td>No</td>
<td>AMKL</td>
</tr>
</tbody>
</table>

MDS = myelodysplastic syndromes, AML = acute myeloid leukemia, CML = chronic myelogenous leukemia, AMKL = acute megakaryocytic leukemia, pre-T LBL = precursor-T lymphoblastic lymphoma/leukemia.

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 differentiatate 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 complementatory approach, we generated transgenic mice on an FVB/N background that express an NHD13 fusion gene under the control of tcf 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 more than 90% of the NHD13 transgenic mice died by 12 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.

Table 2. CBC from NHD13 transgenic mice aged 4–7 months

<table>
<thead>
<tr>
<th>Genotype</th>
<th>WBC*</th>
<th>Neutrophil*</th>
<th>Lymph*</th>
<th>Hgb†</th>
<th>Plt*</th>
<th>BM blasts</th>
<th>BM erythroid dysplasia</th>
<th>BM myeloid dysplasia</th>
<th>BM mega dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenetic</td>
<td>1.8 ± 0.5</td>
<td>0.4 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>11.8 ± 2.1</td>
<td>958 ± 569</td>
<td>14.1 ± 1.9%</td>
<td>12.8 ± 2.1%</td>
<td>6.0 ± 1.3%</td>
<td>Rare</td>
</tr>
<tr>
<td>Control†</td>
<td>6.5 ± 1.8</td>
<td>1.4 ± 0.9</td>
<td>4.8 ± 0.9</td>
<td>14.2 ± 0.7</td>
<td>841 ± 130</td>
<td>5.6 ± 1.8%</td>
<td>1.5±0.9%</td>
<td>1.8 ± 1.0%</td>
<td>Rare</td>
</tr>
</tbody>
</table>

* P value by Mann–Whitney U-test.
† Hemoglobin (Hgb) in g/dL.
‡ White blood cell (WBC), neutrophil, lymphocyte, and platelet (plt) count expressed as 10^9/L.
§ Hematologic malignancy of blast colonies (BL-CFC) (10).
¶ n = 22 for transgenic complete blood count (CBC), n = 7 for control CBC, n = 5 for transgenic bone marrow (BM), and n = 5 for control bone marrow.

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 LBL, with the remainder being nonlymphoid, most commonly myeloid, leukemias. The finding of pre–T LBL in NHD13 mice was unanticipated because human MDS only rarely transforms into a lymphoid malignancy (13,14) and because 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 preexisting 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.

Because 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, seven were euthanized because the mice were sick or moribund and showed signs of MDS but had not transformed to acute leukemia. Of these seven mice, three (#1145, 1899, 1196) had severe anemia (hb < 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 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 white blood cell 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 another 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 and transplantation model, whereas expression of *BCR-ABL* (a “proliferative class” mutation) alone leads to a nonfatal MPD (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, which 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 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 (21) and array-based comparative genomic hybridization (22). We also have begun experiments designed to determine the utility of these mice as a preclinical 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 “premalignant” condition, at least in some cases, can be transplanted before conversion to AML.

### References


Notes
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