

# *NUP98* gene fusions and hematopoietic malignancies: common themes and new biologic insights

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**Structural chromosomal rearrangements of the Nucleoporin 98 gene (*NUP98*), primarily balanced translocations and inversions, are associated with a wide array of hematopoietic malignancies. *NUP98* is known to be fused to at least 28 different partner genes in patients with hematopoietic malignancies, including acute myeloid leukemia, chronic myeloid leukemia in blast crisis, myelodysplastic syndrome, acute lymphoblastic leukemia, and bilineage/biphenotypic leukemia. *NUP98* gene fusions typically encode**

**a fusion protein that retains the amino terminus of *NUP98*; in this context, it is important to note that several recent studies have demonstrated that the amino-terminal portion of *NUP98* exhibits transcription activation potential. Approximately half of the *NUP98* fusion partners encode homeodomain proteins, and at least 5 *NUP98* fusions involve known histone-modifying genes. Several of the *NUP98* fusions, including *NUP98*-homeobox (*HOX*)*A9*, *NUP98*-*HOXD13*, and *NUP98*-*JARID1A*, have been**

**used to generate animal models of both lymphoid and myeloid malignancy; these models typically up-regulate *HOXA* cluster genes, including *HOXA5*, *HOXA7*, *HOXA9*, and *HOXA10*. In addition, several of the *NUP98* fusion proteins have been shown to inhibit differentiation of hematopoietic precursors and to increase self-renewal of hematopoietic stem or progenitor cells, providing a potential mechanism for malignant transformation. (*Blood*. 2011;118(24): 6247-6257)**

## Introduction

One of the oldest, and most useful, whole genome screens for genes involved in malignant transformation is a simple karyotype of the malignant cell.<sup>1</sup> Analysis of recurrent, nonrandom chromosomal translocation breakpoints has identified numerous genes important for malignant transformation and provided critical insight into the biology, classification, and prognosis of hematopoietic malignancies.<sup>2</sup> The study of these genes (such as *BCR-ABL* and *BCL2*) has led to vastly improved therapy<sup>3</sup> and has opened an entire field of scientific inquiry.<sup>4</sup>

The Nucleoporin 98 gene (*NUP98*) was originally identified as a structural component of the nuclear pore complex (NPC),<sup>5</sup> and was subsequently shown to be a fusion partner with homeobox (*HOX*)*A9* in acute myeloid leukemia (AML) patients with a t(7;11)(p15;p15).<sup>6,7</sup> Twenty-eight distinct *NUP98* gene fusions have been identified, caused primarily by balanced translocations and inversions, in the malignant cells of patients with a wide array of distinct hematopoietic malignancies, including AML, chronic myeloid leukemia in blast crisis (CML-bc), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), and bilineage/biphenotypic leukemia.<sup>8</sup> In this overview, we present a summary of the known roles of *NUP98* in normal cell physiology, the association of *NUP98* fusion proteins with hematopoietic malignancies, the incidence and prognostic importance of these fusions, and the mechanisms by which *NUP98* fusion oncoproteins contribute to the process of malignant transformation.

## Normal functions of *NUP98*

### *NUP98* is a component of the NPC

*NUP98* is an ~90-kDa protein component of the NPC, a large multiprotein structure embedded in and traversing the nuclear

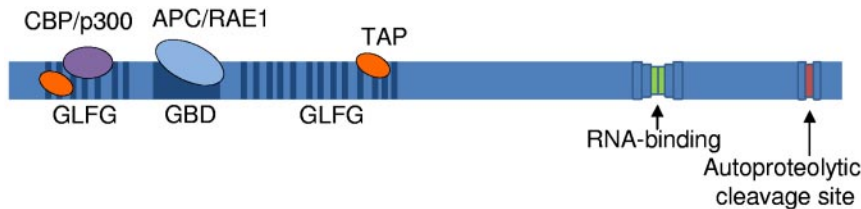
membrane, and consists of ~30 different proteins, many of which are present in multiple copies. *NUP98* has been found on both the nucleoplasmic and cytoplasmic domains of the NPC.<sup>9-11</sup> The NPC provides a bidirectional route of transport between the nucleus and the cytoplasm, allowing small ions and polypeptides to pass through by diffusion and larger macromolecules (mRNA and proteins >40 kDa) by active transport mediated via carrier proteins and transport factors collectively called karyopherins (eg, importins, exportins, and transportin).

The *NUP98* gene encodes 2 alternatively spliced mRNA variants: *NUP98* and *NUP98-NUP96*. *NUP98* and *NUP98-NUP96* precursor polypeptides are cleaved into 90- (N-terminal) and 8- (C-terminal)-kDa peptides and 90- and 96-kDa peptides, respectively.<sup>5</sup> The mature cleaved peptides are generated by *NUP98* autoproteolytic cleavage<sup>12</sup> that is necessary for correct targeting of *NUP98* to the NPC.<sup>5,9,13</sup> (Figure 1).

Approximately a third of all nucleoporin proteins contain repeats of Phe-X-Phe-Gly amino acid residues, or Gly-Leu-Phe-Gly (GLFG) residues, collectively called FG repeats. However, *NUP98* is distinct from other FG nucleoporins in that it contains multiple nontandem GLFG repeats.<sup>11</sup> The GLFG repeats are thought to function as docking sites for karyopherins during trafficking of molecules through the NPC,<sup>11,14</sup> and they have been shown to bind nuclear exportin 1 protein, XPO1 (the human homolog of yeast Crm1)<sup>15,16</sup> and the mRNA export factor TAP.<sup>17</sup>

The nontandem FG repeats of *NUP98* are intersected by a coiled-coil domain, the Gle2-binding sequence (GLEBS) motif in the N-terminal portion of *NUP98* (Figure 1). The GLEBS motif binds the RNA export factor RAE1 (Gle2),<sup>18</sup> and together the RAE1-*NUP98* complex is capable of binding single-stranded

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**Figure 1. Schematic representation of the NUP98 protein.** Blue lines indicate GLFG repeats, the dark blue box indicates the GLEBS binding domain (GBD), and green and red boxes indicate the nucleoporin RNA-binding site and autoproteolytic cleavage site, respectively. Known NUP98 interacting factors are indicated; transcriptional coactivator CBP/p300 (purple) and the TAP transport cofactor (orange) bind the GLFG repeats. The APC/RAE1 complex (light blue) binds the GBD.

RNA,<sup>19</sup> whereas the FG repeats simultaneously bind TAP.<sup>17</sup> The C-terminal end of NUP98 contains an RNA-binding motif.<sup>20-23</sup> As precursor RNA molecules are spliced into their mature forms, they are packaged into messenger ribonucleoprotein particles consisting of these transport and export proteins and delivered to the NPC.

#### NUP98 also is found in the nucleoplasm and is involved in gene transcription

NUP98 was initially believed to function solely as a NPC component and chaperone in the transport and export of messenger ribonucleoprotein particles to the NPC, through the nuclear envelope, and into the cytoplasm. However, it has recently become evident that NUP98 has a broader role in normal cell physiology. NUP98 can be found diffusely throughout the nucleus, largely excluding the nucleolus, in focal clusters called “GLFG bodies.”<sup>15,16</sup> The remarkable mobility of NUP98 seems to be dependent on active transcription by RNA polymerase, although exactly how remains unclear.<sup>15,24</sup> Nup98 seems to function as a transport cofactor for the nuclear export protein Xpo1 (Crm1), interacting with Xpo1 via its GLFG repeats within the GLFG bodies in a Nup98-RanBP3-Xpo1-RanGTP-nuclear export signal cargo protein complex.

The NUP98 GLFG repeats bind CREB-binding protein (CBP)/p300, and a NUP98-GAL4 fusion protein was shown to have transcription activation potential, leading to the speculation that the amino-terminal portion of NUP98 contained a “cryptic” transactivation domain. Direct evidence for the participation of Nup98 in active transcription has recently been demonstrated in fruit flies. Discrete pools of Nup98 protein in the nucleoplasm or the NPC were identified in *Drosophila* cells,<sup>24,25</sup> and the nucleoplasmic Nup98 was found to interact with actively transcribed genes bearing “active” chromatin marks such as H3K4me3, whereas the NPC-associated Nup98 did not associate with these chromatin marks.<sup>25</sup> In addition, Nup98 was colocalized with RNA polymerase (pol) II chromatin “puff” domains<sup>24,25</sup> and decreased levels of Nup98 protein resulted in a decrease in RNA pol II binding and decreased puff formation.<sup>24</sup> Notably, although Nup98 mobility was found to be dependent on RNA pol II activity (active transcription), Nup98 binding of the target sites was not; therefore, Nup98 binding seems to precede active transcription and may play a role in induction of certain genes.<sup>24</sup> Inhibition of Nup98 expression resulted in decreased expression of Nup98 target genes; similarly, overexpression of Nup98 led to increased target gene expression.<sup>25</sup> Nup98 was found to bind specific chromatin sites, and Nup98 protein levels had the most effect on genes involved in cell cycle regulation and differentiation.<sup>24,25</sup> In this context, it is interesting to note that expression of many NUP98 fusion genes impairs differentiation of hematopoietic precursors (see “A role for NUP98 in cell cycle progression and mitotic spindle formation”).

#### A role for NUP98 in cell cycle progression and mitotic spindle formation

In a complex with the Rael protein, Nup98 seems to be involved in mitotic spindle regulation. Dual haploinsufficiency of *Nup98* and

*Rae1* has been shown to result in premature separation of sister chromatids, leading to severe aneuploidy.<sup>26,27</sup> A chromosomal translocation that generates a fusion protein also results in loss of one *NUP98* allele and therefore haploinsufficiency of *NUP98*, but not *RAE1*. However, if the NUP98 fusion protein acted to “sequester” RAE1 via binding to the GLEBS domain of NUP98, RAE1 levels would be functionally decreased, and the fusion protein could, in theory, confer haploinsufficiency of both *NUP98* and *RAE1*. Although speculative, it is possible that aneuploidy resulting from *NUP98* haploinsufficiency may be a mechanism whereby *NUP98* fusion genes could cause genome instability, leading to acquisition of cooperating mutations, progression of disease, and clonal evolution.

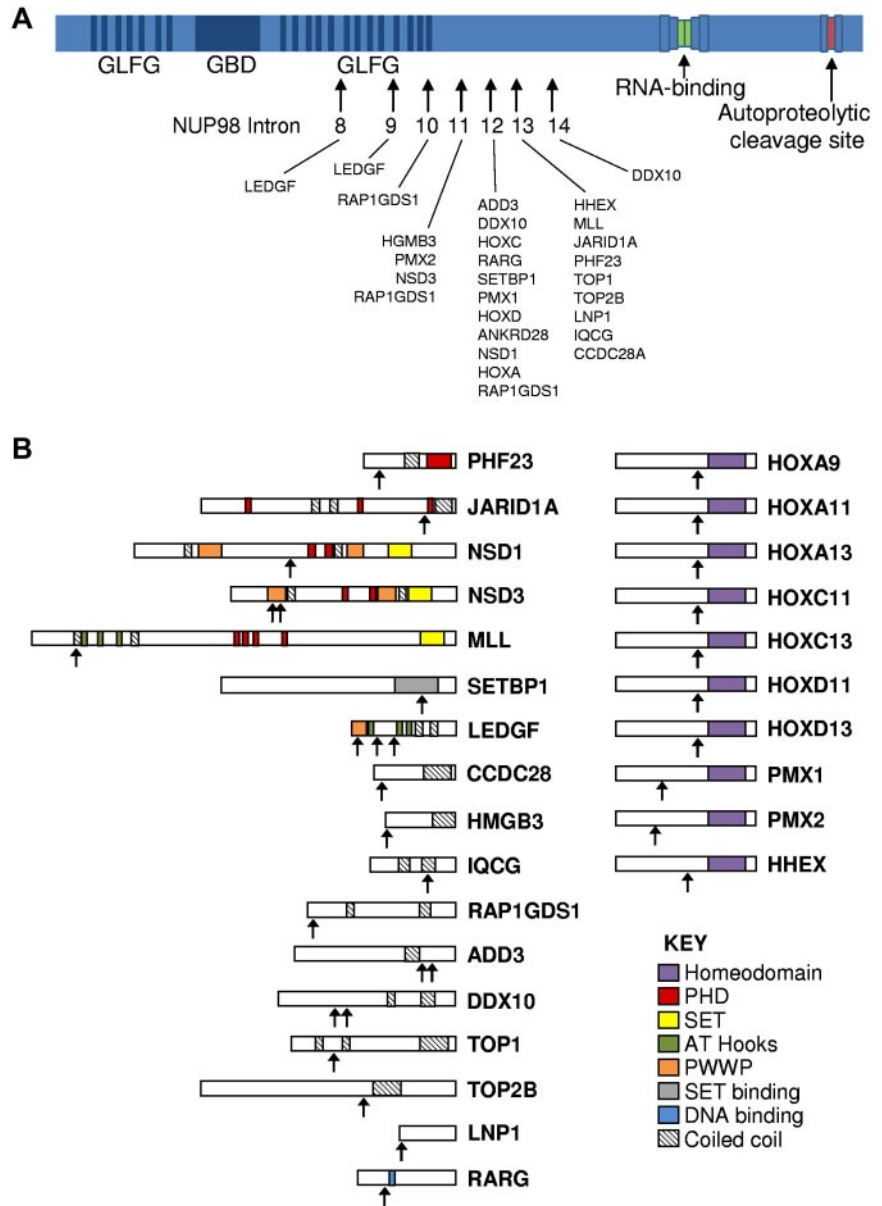
### Chromosomal translocations involving the NUP98 gene occur in a wide range of hematopoietic malignancies

*NUP98* was first linked to hematologic malignancies in 1996, with reports that the t(7;11)(p15;p15) translocation associated with AML generated a fusion gene that encoded the amino-terminal portion of *NUP98* juxtaposed to the carboxyl-terminal portion of *HOXA9*.<sup>6,7</sup> Subsequently, *NUP98* was shown to fuse to numerous partner genes and is now known to produce abnormal fusion proteins with at least 28 different partner genes<sup>8</sup> (Figure 2; Table 1).

It has been difficult to accurately estimate the incidence with which *NUP98* fusions are associated with hematologic malignancy. One study of childhood AML patients from Austria identified one *NUP98* rearrangement in 59 unselected cases,<sup>28</sup> and a study designed to identify solely *NUP98-HOXA9* fusions found 3 in 208 unselected cases of AML in an Asian population.<sup>29</sup> The largest series identified 11 patients with *NUP98-HOXA9* fusions among 493 consecutive AML cases in a Taiwanese population, for a frequency of 2.2%.<sup>30</sup> There have been suggestions that *NUP98-HOXA9* fusions are more common in Asian countries than Western countries, because Asian patients have been overrepresented in published series and case reports of *NUP98-HOXA9* fusions.<sup>29,30</sup> A study by the Groupe Francophone de Cytogenetique Hematologique demonstrated that 35% (23/66) of patients with hematopoietic malignancy and an 11p15 abnormality had a *NUP98* translocation.<sup>31</sup> Taken together, these data suggest that the frequency of *NUP98* rearrangements in unselected patients with AML is 1% to 2% and that the frequency of *NUP98* rearrangements in patients with an 11p15 abnormality is 35%.

*NUP98* fusions are most common in myeloid malignancies, specifically AML, CML-bc, and MDS, but they have not been associated with myeloproliferative neoplasms (MPNs). A recent survey of 96 patients with *NUP98* gene fusions indicated that patients with *NUP98* fusions were relatively young (50% < 20 years) and that 25% of *NUP98* fusions occurred in patients with therapy-related malignancy.<sup>31</sup> Approximately 10% of patients with *NUP98* fusions have T-lineage ALL (T-ALL); most commonly, these malignancies

**Figure 2. NUP98 fusion proteins.** (A) Schematic showing structure of the NUP98 protein and position of NUP98 fusion points in human leukemias. Arrows indicate fusion points. In all cases, the amino terminus of NUP98 is fused to the carboxyl terminus of the partner gene. (B) Schematic showing relevant domains of partner proteins and the position of the protein fusion. Domains are indicated in the key. Arrows indicate fusion point.



are associated with *NUP98-RAP1GDS1* fusions. To date, no B-cell malignancies have been reported to bear a *NUP98* fusion gene. Different partner genes are associated with different diseases, although such associations are rarely exclusive and are based on small numbers of patients. The *NUP98-HOX* gene fusions have not been observed in T-ALL patients and are limited to patients with MDS, AML, juvenile myelomonocytic leukemia, and chronic myelomonocytic leukemia. *NUP98* fusions that have been associated with T-ALL include *ADD3*<sup>32</sup>, *CCDC28A*<sup>31</sup>, *RAP1GDS1*<sup>33</sup> (each of which also have been associated with myeloid malignancies), *IQCG*,<sup>34</sup> and *SETBP1*.<sup>35</sup> Of note, the only reported case of a *NUP98-RARG* fusion was in a patient with promyelocytic leukemia,<sup>36</sup> a leukemia that is almost always associated with a *RARA* fusion gene.<sup>37</sup>

Many *NUP98* fusions are associated with overexpression of *HOXA9*<sup>38,39</sup> that in turn is associated with poor prognosis in AML.<sup>40,41</sup> In addition, AML patients with a *NUP98-HOXA9* fusion have worse overall survival and relapse-free survival.<sup>30</sup> Therefore,

although the patient numbers are small, *NUP98* fusions seem to be associated with poor-prognosis AML.

### NUP98 partner genes

All of the *NUP98* gene fusions thus far identified encode a fusion protein that juxtaposes the amino-terminal portion of NUP98, containing the FG repeats, fused in frame to a partner gene, strongly suggesting that the oncogenic product is the 5'-*NUP98*-partner-3' fusion. The reciprocal 5'-partner-*NUP98*-3' fusion is often, but not invariably, expressed.<sup>42-44</sup>

*NUP98* fusion partners can most simply be divided into 2 categories: homeodomain (HD) proteins and non-HD proteins. HD proteins can be further subdivided into clustered "class I" HD proteins (the HOX proteins) and "class II" nonclustered HD proteins. Ten HD proteins have been demonstrated to be involved in fusions with NUP98, including the most common and best studied of the fusion partners,

**Table 1. NUP98 fusion partner genes**

Partner gene	Chromosome	Disease	Relevant domain(s)	Reference(s)
<i>HOXA9</i>	7p15	MDS, AML, CML, CMML	HD	6,7
<i>HOXA11</i>	7p15	MDS, AML, CML, JMML	HD	135
<i>HOXA13</i>	7p15	AML	HD	136
<i>HOXC11</i>	12q13	AML	HD	137
<i>HOXC13</i>	12q13	AML	HD	138
<i>HOXD11</i>	2q31	AML	HD	139
<i>HOXD13</i>	2q31	MDS, AML, CML	HD	42
<i>PMX1</i>	1q23	AML, CML	HD	140
<i>PMX2</i>	9q34	AML	HD	141
<i>HHEX</i>	10q23	AML	HD	58
<i>PHF23</i>	17p13	AML	PHD, CC	142
<i>JARID1A</i>	12p13	AML	PHD, CC	143
<i>NSD1</i>	5q35	MDS, AML, T-ALL	PHD, CC, SET	144
<i>NSD3</i>	8p11	MDS, AML	PHD, CC, SET	145
<i>MLL</i>	11q23	AML	PHD, CC, SET	121
<i>SETBP1</i>	18q12	T-ALL	CC	35
<i>LEDGF</i>	9p22	AML, CML	CC	43
<i>CCDC28</i>	6q24	AML, T-ALL	CC	146
<i>HMGB3</i>	Xq28	AML	CC	147
<i>IQCG</i>	3q29	T-ALL	CC	34
<i>RAP1GDS1</i>	4q21	AML, T-ALL	CC	33
<i>ADD3</i>	10q25	AML, T-ALL	CC	32
<i>DDX10</i>	11q22	MDS, AML, CML, CMML	CC	148
<i>TOP1</i>	20q11	MDS, AML	CC	149
<i>TOP2B</i>	3p24	AML	CC	150
<i>LNP1</i>	3q12	AML	CC	151
<i>RARG</i>	12q13	AML	CC	36
<i>ANKRD28</i>	3p25	AML	Ankyrin	152

CMML indicates chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; and CC, coiled-coil.

*HOXA9* (Figure 2; Table 1). The class II nonclustered HD genes involved in *NUP98* fusions are *HHEX*, *PMX1*, and *PMX2*. In all instances, the C-terminal DNA-binding HD of the HD protein is retained in the fusion protein, and the transactivation domain is replaced by the GLFG repeats of *NUP98*.

Eighteen of the known *NUP98* fusion partners do not encode HDs. Several of these genes lack a known DNA-binding domain,

suggesting that they might have a mode of action distinct from that of the HD fusions. Although initially the non-HD partner genes were thought to have no common structural motifs, some patterns have emerged as more partner genes have been identified. The majority of these genes encode proteins that contain a coiled-coil domain that is thought to function in oligomerization of proteins (determinations made using the COILS 2.0 server<sup>45,46</sup>).

In addition to the coiled-coil domains, a recurring theme among some of the non-HD fusion partners is the presence of a histone “reading” or “writing” domain. In particular, 5 fusion partner genes encode plant HD (PHD) fingers, chromatin recognition domains that have been shown, in at least some cases, to be required for the leukemogenic potential of the fusion<sup>47</sup> (Table 1). Three of these genes (*NSD1*, *NSD3*, and *MLL*) also encode suppressor of variegation-enhancer of zeste-trithorax (SET) domains, which have a histone methyltransferase function. Indirectly, this theme is maintained for SETBP1 that interacts with a SET domain-containing protein. These proteins may comprise a third class of *NUP98* fusion partner gene, with a specific mechanism of leukemogenesis (see “Proving oncogenicity: mouse models of *NUP98* fusions”).

## Proving oncogenicity: mouse models of *NUP98* fusions

Mouse models of leukemia initiated by a wide range of leukemic fusion genes have been shown to recapitulate key features of the human malignancies in which they have been found, and they generally use 1 of 2 approaches. In the first, wild-type bone marrow cells transduced with a retrovirus encoding the leukemic fusion gene are transplanted into lethally irradiated recipients. An alternate approach is to generate genetically engineered (either transgenic or “knockin”) mice that express the fusion gene in the hematopoietic compartment. Both approaches have been used to study *NUP98* fusion genes (Table 2).

### *NUP98*-HOX fusions

Expression of the *NUP98*-*HOXA9* fusion via bone marrow transduction and transplantation led to an MPN characterized by increased white blood cells, anemia, and decreased T- and B-cell progenitors.<sup>48</sup> After a latency of at least 4 months, 11 of 14 mice progressed

**Table 2. Murine models of NUP98 fusions**

Fusion gene	Phenotype	Method	Reference(s)
<i>NUP98</i> - <i>HOXA9</i>	MPN progressing to AML (mean latency, 230 d)	BMT	48
<i>NUP98</i> - <i>HOXA9</i>	MPN progressing to AML (mean latency, 450 d)	Transgenic	49
<i>NUP98</i> - <i>HOXA9</i> and <i>Meis1</i>	MPN progressing to AML (mean latency, 142 d)	BMT	48
<i>NUP98</i> - <i>HOXA9</i> and <i>BCR-ABL</i>	AML (latency, 21 d)	BMT	123
<i>NUP98</i> - <i>HOXA9</i> and <i>TEL-PDGFβR</i>	AML (latency, 25 d)	BMT	123
<i>NUP98</i> - <i>HOXA10</i>	MPN progressing to AML (mean latency, 223 d)	BMT	50,51
<i>NUP98</i> - <i>HOXA10</i> and <i>Meis1</i>	MPN progressing to AML (mean latency, 70 d)	BMT	50,51
<i>NUP98</i> - <i>HOXD13</i>	MPN, not progressing to AML	BMT	38
<i>NUP98</i> - <i>HOXD13</i> and <i>Meis1</i>	MPN progressing to AML (median latency, 75 d)	BMT	38
<i>NUP98</i> - <i>HOXD13</i>	MDS progressing to AML (median latency, 9 mo)	Transgenic	54,55
<i>NUP98</i> - <i>PMX1</i>	MPN (mean latency, 246 d)	BMT	57
<i>NUP98</i> - <i>PMX1</i> and <i>Meis1</i>	MPN progressing to AML (median latency, 191 d)	BMT	57
<i>NUP98</i> - <i>HHEX</i>	AML (mean latency, 9 mo)	BMT	58
<i>NUP98</i> - <i>TOP1</i>	AML (mean latency, 233 d)	BMT	59
<i>NUP98</i> - <i>TOP1</i> and <i>Meis1</i>	AML (mean latency, 242 d)	BMT	59
<i>NUP98</i> - <i>NSD1</i>	AML (mean latency, 126 d)	BMT	39
<i>NUP98</i> - <i>JARID1A</i>	AML (mean latency, 69 d)	BMT	47

BMT indicates retroviral bone marrow transduction.

to AML. Secondary recipients demonstrated that the disease was transplantable, and coexpression of the *Hox* cofactor *Meis1* reduced the latency to onset of AML. *NUP98-HOXA9* transgenic mice showed comparable disease, with an MPN that transformed into AML in 22% of cases after a long latency of 15 months.<sup>49</sup> Transduction of bone marrow cells with *NUP98-HOXA10* produced a similar phenotype, with an MPN progressing to AML after a prolonged latency, that could be accelerated by cotransduction of *Meis1*.<sup>50,51</sup> Surprisingly, coexpression of an *Meis1* DNA-binding mutant also accelerated AML conversion, suggesting that the action of *Meis1* may not be dependent on DNA binding.<sup>50,51</sup>

Expression of *NUP98-HOXD13* by transduction of primary murine bone marrow cells also resulted in increased proliferation of immature myeloid cells *in vitro*.<sup>38</sup> At 6 to 8 months after transplantation, several of the *NUP98-HOXD13* recipients developed MPN, and several others developed severe anemia with T and B lymphopenia; coexpression of *Meis1* led to rapid development of AML. *Hoxa7* and *Hoxa9* transcript levels were elevated in both the *NUP98-HOXD13* and *NUP98-HOXD13/Meis1* transplant recipients, although endogenous levels of *Meis1* were not elevated.

Transgenic mice that expressed a *NUP98-HOXD13 (NHD13)* fusion under control of pan-hematopoietic *Vav* regulatory elements developed an MDS characterized by peripheral blood cytopenias, ineffective hematopoiesis, dysplasia, and apoptosis.<sup>52-54</sup> Approximately 60% of the mice develop AML; the remaining mice died of severe pancytopenia or developed T-ALL or B-lineage ALL.<sup>54,55</sup> (S.M.G. and P.D.A., unpublished data, 2011). The lymphoid leukemias that developed in these mice demonstrated that expression of the *NUP98-HOX* fusions could be leukemic in lymphoid as well as myeloid cells. Bone marrow replating assays demonstrated increased repopulating abilities of the NHD13 marrow compared with wild-type marrow, similar to the findings with other *NUP98-HOX* fusions described under “NUP98-HOX fusions.” Lymphopoiesis in clinically healthy *NHD13* mice was found to be partially blocked at the DN2-DN3 transition in thymocytes and at the pro-B to pre-B transition in B cells.<sup>56</sup> *Hoxa5*, *Hoxa7*, *Hoxa9*, and *Hoxa10* levels were markedly elevated in both the bone marrow and thymus; however, *Meis1* mRNA levels were decreased compared with those found in wild-type bone marrow or thymus.<sup>53,56</sup> Limiting dilution assays of *NHD13* bone marrow identified the presence of a clonal transplantable MDS-initiating cell.<sup>53</sup>

Expression of a *NUP98-PMX1* fusion resulted in MPN, with 50% of the mice dead by 300 days posttransplantation.<sup>57</sup> *NUP98-PMX1* enhanced myeloproliferation, impaired lymphopoiesis, and collaborated with *Meis1* during AML transformation. These abilities were shown to be dependent on both the GLFG repeats of *NUP98* and the DNA-binding activity of the *PMX1* HD. Similar to the *NUP98-HOX* fusions, *NUP98-PMX1* induced expression of the *Hoxa* cluster genes (*Hoxa-5*, *-7*, *-9*, and *-10*) that was dependent on the direct binding of the fusion protein to *Hoxa* regulatory regions.<sup>57</sup>

The *NUP98-HHEX* fusion also conferred aberrant self-renewal and impaired differentiation, properties that are dependent on the combined integrity of both the *NUP98* FG repeats and the HD of *HHEX*.<sup>58</sup> *HHEX* normally acts as a transcriptional repressor; however, its N-terminal repressive elements are replaced with the transactivating FG repeats of *NUP98*, transforming the *HHEX* repressive function into one of transcriptional activation. Bone marrow transplant of cells that expressed a *NUP98-HHEX* fusion resulted in AML that was associated with up-regulation of *Hoxa5*, *Hoxa9*, and *Fli3*.<sup>58</sup>

### NUP98 non-HOX fusions

Expression of the *NUP98-TOP1* fusion protein in a bone marrow transplant model led to MPN and AML.<sup>59</sup> Similar to the *NUP98-HOX* fusions, both the *NUP98* GLFG repeats and the *TOP1* DNA-binding domain were essential for leukemogenesis; interestingly, leukemic transformation was independent of the topoisomerase activity of *TOP1*. In contrast to *NUP98-HOX* fusions, AML transformation was not accelerated by *Meis1*, suggesting the possibility that some aspect of transformation caused by the *NUP98-TOP1* fusion is unique to this gene fusion.

Chromatin-modifying *NUP98* fusions include *NUP98-NSD1*, *NUP98-JARID1A*, and *NUP98-PHF23*, and they may encode a distinct class of fusion oncoproteins. These fusions alter the conformation of chromatin by binding histone tails and “writing” or “reading” the active marks, thus modifying normal gene regulation. *NUP98-NSD1*-transduced bone marrow cells increased the expression of the *Hoxa5*, *a7*, *a9*, *a10*, and *Meis1* and resulted in AML.<sup>39</sup> *NUP98-NSD1* was shown to bind and activate the promoters of *Hoxa7* and *Hoxa9* and was dependent on a PHD finger within the *NSD1* portion of the fusion. Full transforming potential required the combination of the *NUP98* GLFG repeats for recruitment of the CBP/p300 histone acetylation complex, the H3K36 methyltransferase activity of the *NSD1* SET domain,<sup>60</sup> and the binding of the *Hoxa* locus via the PHD domain. The *NUP98-NSD1* fusion thus behaves as a potent transcriptional activator at the *Hoxa* locus by bringing together 2 epigenetic modifiers that prepare chromatin for active gene transcription, alongside a PHD domain that targets the *Hoxa9* promoter.<sup>39</sup> These findings support a model in which the *NUP98-NSD1* fusion maintains a zone of active transcription by enforcing epigenetic modification at *Hoxa* gene regulatory elements while simultaneously preventing epigenetic silencing and repression by the polycomb repressor complex that normally silences the *Hoxa* locus during hematopoietic differentiation.

The epigenetic changes as just described also occur with *NUP98-JARID1A* and *NUP98-PHF23* fusion proteins.<sup>47</sup> Both fusions show transforming potential by enhancing progenitor cell self-renewal and arresting myeloblast differentiation. Mice transplanted with bone marrow cells transduced with the *NUP98-JARID1A* fusion developed AML, and transformation was shown to be dependent on the integrity of the PHD fingers that were determined to specifically bind di- or trimethylated H3K4 (H3K4me2/3). ChIP experiments showed binding of the *NUP98-JARID1A* fusion increased the expression of *Hoxa5*, *a7*, *a9*, and *a10* genes. Mutation of conserved residues in the PHD fingers abrogated H3K4me3 binding, leading to failure of the fusion proteins to bind the *Hoxa9* promoter, no change in *Hoxa9* expression levels, and no leukemic transformation. Thus, the *NUP98-JARID1A* fusion represents a deregulated “reader” of histone marks and effectively blocks the epigenetic program required for normal cellular differentiation by maintaining expression of the *Hoxa* gene cluster.

Despite some degree of variation, potentially because of either the model system used or to the specific partner gene, several common themes can be observed from these *in vivo* models of *NUP98* fusion genes. Anemia and aberrant differentiation of the myeloid lineage, with either increased or decreased circulating granulocytes is present, and some of the models have reported impaired T- and B-cell differentiation. Marked overexpression of *Hoxa* cluster genes, most notably *Hoxa5*, *a7*, *a9*, and *a10*, is associated with the leukemic phenotype, and transformation to

AML can be accelerated by coexpression of *Meis1* in many cases. Both fusion partners possess protein domains essential for leukemogenesis, and collaborating mutations are likely to be required for evolution from a “preleukemic” MPN or MDS to AML.

## Mechanism(s) of leukemic transformation initiated by *NUP98* fusion genes

### *NUP98* fusion genes encode aberrant transcription factors

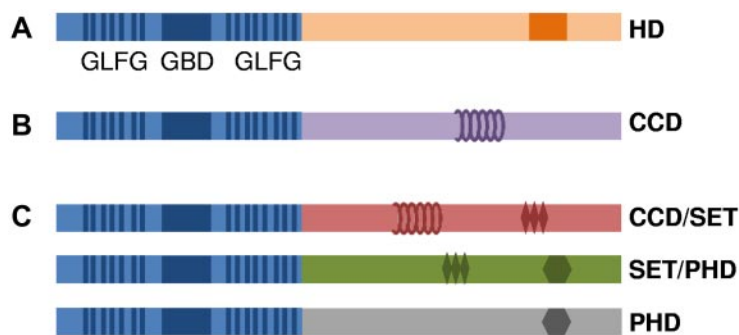
Several lines of evidence suggest that many of the *NUP98* fusion proteins act as aberrant transcription factors. Although the wild-type *NUP98* protein is primarily localized to the NPC, *NUP98* fusion proteins are predominantly located in the nucleoplasm. The *NUP98-HOXA9*, *NUP98-HOXD13*, *NUP98-TOP1*, *NUP98-PMX1*, *NUP98-NSD1*, *NUP98-IQCG*, *NUP98-PHF23*, *NUP98-JARID1A*, *NUP98-DDX10*, and *NUP98-HHEX* fusion proteins have all been shown to be widely dispersed throughout the nucleus and in punctate foci called GLFG bodies in the nucleus, with the exception of the nucleoli.<sup>34,39,47,58,61-64</sup> Although leukemogenicity has been shown to be dependent on the GLFG repeats that recruit the transcriptional coactivator complex CBP/p300,<sup>39,61,64,65</sup> the interaction of *NUP98* with RAE1 is not required for leukemic transformation.<sup>65</sup>

Furthermore, *NUP98* fusion partner genes often contain domains known to be involved in gene transcription. Fusion partners seem to fall into 3 principal groups that either (1) directly bind DNA and activate gene transcription (HD transcription factors), (2) facilitate protein–protein interactions with transcriptional cofactors involved in active gene transcription (coiled-coil domains), or (3) modify chromatin to initiate gene transcription, maintain gene transcription, or both, gene transcription by “writing” or “reading” active chromatin marks (Figure 3). Coiled-coil domains have been shown to be necessary for the transforming abilities of the PML-RAR, AML1-ETO, and PAX-PML<sup>66,67</sup> fusion proteins, and the proteins encoded by *NUP98* partner genes *DDX10*, *RAP1GDS1*, *LEDGF*, *TOP1*, and *NSD1* have been predicted or demonstrated<sup>45,68</sup> to form coiled-coil motifs that can mediate protein–protein interactions.

Several *NUP98* partner genes encode fusion proteins that contain PHD domains, SET domains, or both. As discussed already, the *NUP98-NSD1* fusion protein has been shown to activate expression of *Hoxa5*, *a7*, *a9*, *a10*, and the Hox transcriptional cofactor *Meis1*.<sup>39</sup> Chromatin modification was evident around the *Hoxa7/a9* loci in cells that expressed a *NUP98-NSD1* fusion compared with wild-type progenitor cells.<sup>39</sup> In these cells, the *Hoxa7/a9* locus was strongly H3 acetylated, displayed H3K36me3 marks, lacked H3K27me3 marks, and was bound by p300, all

features that are consistent with active gene transcription.<sup>39</sup> Three functionally distinct domains of the *NUP98-NSD1* fusion protein were required for transcriptional activation of the *Hoxa* locus and immortalization of myeloid progenitor cells; the fifth PHD finger of NSD1, the SET domain of NSD1, and the *NUP98* GLFG repeats. The *NUP98-NSD1* fusion protein bound to chromatin 5′ of *Hoxa7* and *Hoxa9* via the PHD domain, recruited p300/CBP via the FG repeats, and methylated H3K36 via the SET domain, resulting in active gene transcription.<sup>39</sup> Similar observations were made for the *NUP98-JARID1A* and *NUP98-PHF23* fusions.<sup>47</sup> In those studies, *Hoxa5-a11* genes were overexpressed, and active H3K4me3 histone marks were localized to *Hoxa5-a11*, whereas flanking transcriptionally silent domains were marked by H3K27me and associated with the polycomb repressor complex components Suz12 and Ezh2.<sup>47</sup> Deletion mutants of the PHD domains of *JARID1A* and *PHF23* in the fusion proteins showed that the PHD fingers, which recognize H3K4me2/3, were essential for leukemic transformation. Binding of the PHD finger to H3K4me2/3 correlated with an open and active domain of gene transcription, suggesting that the fusion proteins prevented the spreading of H3K27me that is associated with silencing across the *Hoxa* locus during normal cell differentiation and maturation.

Given that some of the *NUP98* fusions function as transcriptional regulators, several investigators have used gene expression arrays to identify genes and pathways regulated directly or indirectly by *NUP98* fusion proteins. Analysis of *NUP98-HOXA9* expression in human CD34<sup>+</sup> cells using the Affymetrix U133+2.0 platform revealed up-regulation of interferon pathway genes (*IFI44*, *IFI44L*, *IFIT1*, *MX1*, and *OAS1*), *HOX* genes (*HOXA3*, *HOXA5*, and *HOXA9*), genes involved in myeloid differentiation (*ELA2A* and *CTSG*), and *REN* that encodes renin, a protease best known for its role in blood pressure regulation.<sup>69</sup> A similar experiment using cord-blood CD34<sup>+</sup> cells transduced with a *NUP98-HOXA9* fusion and the Affymetrix U133A platform demonstrated up-regulation of *HOX* genes (*HOXA5*, *HOXA6*, *HOXA7*, *HOXA9*, and *MEIS1*), as well as *RUNX1*, *CD44*, *PIM1*, and *HLF1*.<sup>70</sup> Expression of a *NUP98-HOXA10* or *NUP98-HOXD13* fusion in murine stem and progenitor cells led to overexpression of *Crispl*, *Nr4a1*, *Hlf1*, *Ahr*, *Pbx3*, *Hoxa5*, and *Hoxa7*.<sup>71</sup> Comparison of bone marrow from *NUP98-HOXD13* transgenic mice to bone marrow from wild-type mice using a MEEBO/Stanford array demonstrated that 3 of the 10 most highly overexpressed genes were interferon-induced (*Oas2*, *Ifit1*, and *Ifi44*), and 4 encoded HD proteins (*Hoxa7*, *Hoxa9*, *Hoxc6*, and *Pbx3*; C.S. and P.D.A., unpublished data, 2007). However, in contrast to findings with other *NUP98* fusion proteins, expression of the *NUP98-HOXD13* fusion did not lead to up-regulation of *Meis1* in that experiment. Expression of a *NUP98-DDX10* fusion in human CD34<sup>+</sup> cells demonstrated that the most



**Figure 3. Schematic representation of 3 archetypes of *NUP98* fusions.** (A) *NUP98-HD* fusion protein. (B) *NUP98*-coiled-coil motif fusion protein. (C) Three subgroups of *NUP98* oncoproteins that potentially modify chromatin via coiled-coil domain (CCD), SET, or PHD finger motifs. The GBD is represented by a blue box, the GLFG repeats are shown as vertical blue lines, the HD domain is shown as an orange box, the CCD is shown as coils, the SET domain is shown as diamonds, and the PHD domain is shown as a hexagon.

up-regulated genes were *HOXA* cluster, *MEIS1*, and *REN*.<sup>63</sup> Taken together, the data demonstrate that genes that encode HD proteins, particularly *HOXA* cluster genes, *MEIS1* and *PBX3*, are typically up-regulated by expression of NUP98 fusion proteins, as are a group of interferon-inducible genes.

#### NUP98 fusion proteins deregulate *HOXA* cluster genes

The clustered *HOX* genes (class I *HOX* genes) are a family of genes that encode a conserved HD motif and function as DNA-binding transcription factors. The *HOX* genes were initially identified as “master regulators” of positional identity and body-pattern formation in *Drosophila melanogaster* (reviewed in McGinnis and Krumlauf<sup>72</sup>). Mammals have 39 *HOX* genes, organized in 4 discrete clusters designated A to D.<sup>73-75</sup> A larger group of ~160 less-conserved, nonclustered homeobox genes (class II *HOX* genes) are dispersed throughout the genome (reviewed in Argiropoulos and Humphries<sup>73</sup> and Lawrence et al<sup>76</sup>).

Expression of *Hoxa* cluster genes is important for normal hematopoietic differentiation (reviewed in Argiropoulos and Humphries<sup>73</sup>). Although all 11 of the *Hoxa* cluster genes are expressed in murine HSCs, the most highly expressed and differentially regulated genes are *Hoxa5*, *a7*, *a9*, and *a10*.<sup>77</sup> In general, these 4 *Hoxa* genes are most highly expressed in the hematopoietic stem and progenitor cells (HSPCs), and they seem to regulate HSPC self-renewal and repopulation of early myeloid and lymphoid progenitors.<sup>78-83</sup> As progenitor cells proliferate and differentiate, thus losing their self-renewal properties, these 4 *Hoxa* cluster genes are down-regulated, as is the *Hox* cofactor *Meis1*.<sup>78,79,84</sup>

Approximately 50% of unselected human AML samples overexpress *HOXA* cluster genes, particularly *HOXA7*, *A9*, and *A10*, often in conjunction with the binding partner *MEIS1*.<sup>40,71,84-88</sup> In addition, overexpression of *HOXA9* has been linked to poor clinical outcome; indeed, a gene expression array study identified overexpression of *HOXA9* as the single gene that most correlated with treatment failure in AML patients.<sup>41</sup> Furthermore, several specific cytogenetic and molecular genetic AML subtypes overexpress *HOXA* cluster genes, including those with *MLL*-fusions,<sup>40,89,90</sup> *CALM-AF10* fusions,<sup>91</sup> monosomy 7,<sup>92</sup> and *NPM1* mutants.<sup>93-95</sup> Therefore, although NUP98 fusions are rare, they typically deregulate expression of *HOXA* cluster genes, particularly *HOXA7*, *A9*, and *A10*, whose overexpression is commonly associated with acute leukemia.

As we have described, expression of *Meis1* was shown to enhance the leukemogenicity of NUP98 fusion proteins as well as *Hoxa9*<sup>96</sup>, and *MEIS1* overexpression is often associated with overexpression of *HOXA7* and *HOXA9* in AML patients.<sup>71,86</sup> *MEIS1* and the *PBX1*, *PBX2*, and *PBX3* proteins are members of the “TALE” (3-amino acid loop extension) family of transcriptional cofactors that bind HOX proteins and serve to increase target specificity and stabilize DNA binding.<sup>97-101</sup> *Meis1* rather than the *Pbx1* seems to be the more crucial cofactor for the transformation of myeloid bone marrow cells, because cotransduction of *Meis1* and *Hoxa9* resulted in AML in mice and cotransduction *Pbx1* and *Hoxa9* did not.<sup>102</sup> A recent report indicates that levels of *Meis1* may be the rate-limiting factor for leukemic transformation of *MLL*-fusion proteins, because higher levels of *Meis1* correlated with reduced latency of disease.<sup>103</sup> This also may be the case with NUP98-*HOX* fusions.

Of note, the microRNA (miR) miR-196b is located between *HOXA9* and *HOXA10* in both the mouse and human genomes. Small regulatory microRNAs play a major role in the regulation of gene expression (reviewed in Taft et al<sup>104</sup> and Zhao et al<sup>105</sup>), and

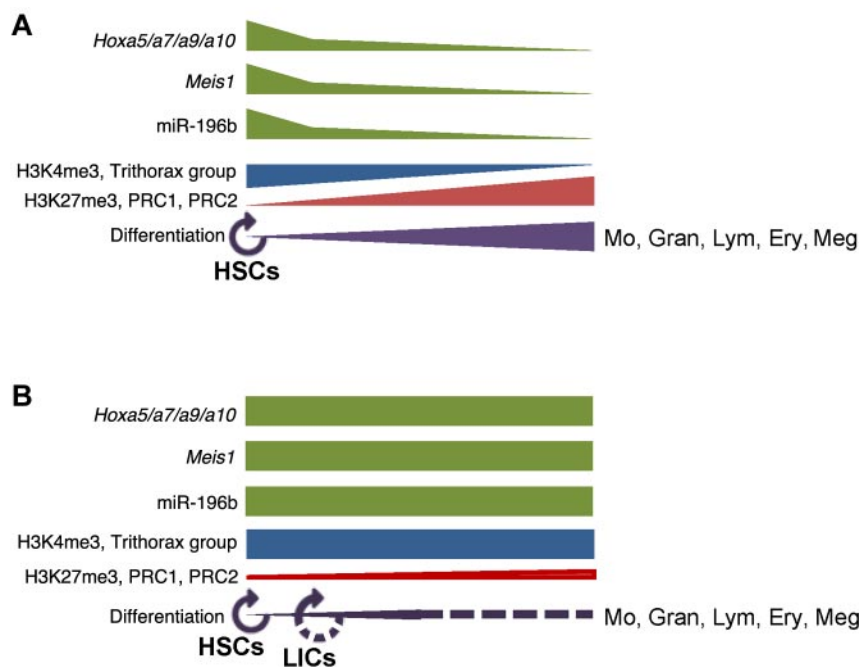
have now been profiled in leukemia samples to elucidate expression patterns that might signify specific genomic alterations, leukemia subtype classification, and treatment response.<sup>106-111</sup> miR-196b is more highly expressed in progenitor than in differentiated hematopoietic cells, and it is thought to play a role in the regulation of normal hematopoiesis.<sup>112,113</sup> Expression of miR-196b and *HOXA9* and *HOXA10* is highly correlated in leukemia samples,<sup>114</sup> leading to the suggestion that miR-196b may be cotranscribed with the *HOXA* cluster.<sup>112,115</sup> NUP98-*HOXD13* and NUP98-*PHF23* transgenic mice both overexpress miR-196b relative to wild-type bone marrow, and a transcript that contains both miR-196b and *Hoxa9* sequences has been identified (S.M.G. and P.D.A., unpublished data, 2011), supporting the hypothesis that the *Hoxa* cluster is “open” to transcription in NUP98 fusion AMLs.

The mechanism(s) by which overexpression of *HOXA* cluster genes leads to leukemic transformation is unclear. However, given that expression of several *HOXA* cluster genes is down-regulated as stem cells mature, it is feasible that persistent expression of *HOXA* cluster genes enforces an immature stem or progenitor phenotype. HSC self-renewal is normally tightly controlled, and stem cells must balance self-renewal with generation of committed progenitors, so that sufficient numbers of committed progenitors are produced to sustain mature hematopoiesis. This balance is thought to be achieved through a combination of both asymmetric and symmetric HSC division, in which asymmetric division of HSCs gives rise to one committed daughter cell and one “self-renewal” cell. In contrast, symmetric division of HSCs gives rise to 2 self-renewing HSCs, leading to expansion of HSC number (reviewed in Morrison and Kimble<sup>116</sup>). Of note, although overexpression of *BCR-ABL* primarily increased the rate of hematopoietic precursor cell division, expression of NUP98-*HOXA9* in murine cells led to increased symmetric division of hematopoietic precursors.<sup>117</sup> In addition, enforced expression of a NUP98-*HOXA9* fusion in human CD34<sup>+</sup> cells led to expansion of HSCs, as measured by cobblestone formation and competitive repopulation assays.<sup>70</sup> Taken together, these data indicate that expression of NUP98-*HOXA9* can expand the pool of self-renewing, undifferentiated HSCs (Figure 4).

#### Events that collaborate with NUP98 fusion genes during leukemic transformation

It is generally accepted that mutations of a single gene are rarely sufficient to cause malignant transformation but that instead several collaborative mutations are required.<sup>118,119</sup> A working framework for leukemic transformation has emerged in which one mutation from each of 2 distinct classes (“type I” and “type II”) is required.<sup>120</sup> Type I mutations lead to increased proliferation, survival, or both, whereas type II mutations impair differentiation or enhance self-renewal of HSC/progenitor cells. The NUP98 fusions that have been studied seem to fall into the type II category, based on the ability of the NUP98-*HOXD13* fusion<sup>54</sup> to block 12-*O*-tetradecanoylphorbol-13-acetate-induced differentiation of K562 cells, and the impaired ability of HSCs and progenitor cells from NUP98-*HOXD13* transgenic mice to differentiate in vitro.<sup>52</sup> Recent work has indicated that this framework may be overly simplified, especially for the fusions such as NUP98-*JARID1A*, NUP98-*PHF23*, and NUP98-*MLL* that are known or suspected to function by modifying chromatin at numerous regions genome-wide,<sup>47,121</sup> potentially affecting numerous genes with diverse functions.

The contention that some NUP98 fusions, such as the NUP98-*HOX* fusions, are type II mutations is supported by the



**Figure 4. Simplified model of the relationship between *Hoxa* cluster gene expression and epigenetic modifications.** (A) During normal hematopoiesis, *Hoxa* and miR-196b transcript levels are highest in HSPCs and decrease as cells differentiate to functional mature hematopoietic cells. In immature HSPCs, active histone marks such as H3K4me3 and the presence of trithorax group proteins correlate with high levels of *Hoxa* transcripts. These transcriptional enhancer marks decrease as cells mature and differentiate, and the *Hoxa* locus becomes progressively silenced by repressive H3K27me3 marks and polycomb repressor complex (PRC) group proteins (129,130-134; [130-134 are reviews]). (B) Misregulated *Hoxa* cluster gene expression associated with *NUP98* fusion leukemogenesis, as a result of aberrant chromatin-modifying activities conferred by the *NUP98* fusion.<sup>39,47</sup> Mo indicates monocytes; Gran, granulocytes; Lym, lymphocytes; Ery, erythrocytes; Meg, megakaryocytes; and LICs, leukemia-initiating cells.

identification of collaborating events in *NUP98-HOX* fusion leukemias, because most of the described collaborating events are type I proliferation and survival mutations. *NUP98-HOXA9* fusions have been identified in patients with Ph+ CML-bc,<sup>122</sup> and the collaborative nature of *NUP98-HOXA9* and *BCR-ABL* fusions was confirmed in an experimental mouse model.<sup>123</sup> Of 27 patients with *NUP98* fusions (predominantly *NUP98-HOX* fusions), no mutations of *MLL*, *NPM1*, *RUNX1*, or *CEBPA* (all type II mutations) were identified; however, 25 type I events in total were found, including the *FLT3*-ITD mutation (12) and activating mutations in *NRAS* (5), *KRAS* (4), and *KIT* (4).<sup>30,124</sup>

A study of the complementary mutations that arise in leukemias of *NUP98-HOXD13* transgenic mice supported the findings from human patients.<sup>125</sup> In a screen of 32 leukemias, no type II mutations were identified (*Npm1* and *Runx1* were screened), whereas almost one third of the mice had acquired a spontaneous type I mutation (4 *Nras*, 5 *Kras*, and 1 *Cbl*). None of the *NUP98-HOXD13* mice with MDS that had not transformed to AML had evidence of a *Nras* or *Kras* mutation, suggesting that the *Nras* and *Kras* mutations are associated with progression of MDS to AML. Although *Flt3* mutations did not occur spontaneously in that series, a recent study showed that the *FLT3*-ITD mutation markedly accelerates the onset of AML in *NUP98-HOXD13* transgenic mice (S. Greenblatt and D. Small, personal e-mail communication, July 11, 2011), and another demonstrated that overexpression of the wild-type *FLT3* accelerates the onset of AML in mice that express *NUP98-HOXD13*.<sup>126</sup>

Retroviral insertional mutagenesis has been used as a tool to identify collaborating mutations in several *NUP98* fusion mouse models. One such study is a *NUP98-HOXA9* model crossed onto a BXH2 background that identified cooperating events such as *Meis1* and genes that encoded dynein motor complex proteins.<sup>49</sup> A second mutagenesis screen performed on *NUP98-HOXD13* mice identified *Meis1* overexpression and *Mn1* overexpression as important complementary events,<sup>127</sup> and a third study identified inactivation of *ICSBP* as a complementary event for *NUP98-TOP1* expression.<sup>128</sup>

## Summary

The *NUP98* protein has several distinct roles within the nucleus of the normal cell. Fusion of *NUP98* to a large number of “partner genes” leads to the generation of leukemogenic *NUP98* fusion proteins. *NUP98* fusions are associated with a wide spectrum of hematopoietic malignancies, including MDS, AML, CML-bc, and pre-T lymphoblastic lymphoma. It is not yet clear whether this diversity reflects the *NUP98*-partner gene, the cell type that undergoes the initial *NUP98* fusion event, or a combination of both. Complementary mutations frequently recognized in *NUP98* fusion leukemias include genes that encode proteins involved in proliferation, resistance to apoptosis, or both, such as *BCR-ABL*, *NRAS*, *KRAS*, and *FLT3*. A common theme that emerges among many of the leukemias that express *NUP98* fusion proteins is deregulation of the *HOXA* gene cluster, leading to impaired terminal differentiation of hematopoietic cells. The fact that deregulation of *HOXA* cluster gene expression is a frequent finding in leukemias initiated by *NUP98* fusions make this cluster of genes an attractive target for novel therapeutic approaches.

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