Review

Nuclear pore proteins and cancer

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Nucleocytoplasmic trafficking of macromolecules, a highly specific and tightly regulated process, occurs exclusively through the nuclear pore complex. This immense structure is assembled from approximately 30 proteins, termed nucleoporins. Here we discuss the four nucleoporins that have been linked to cancers, either through elevated expression in tumors (Nup88) or through involvement in chromosomal translocations that encode chimeric fusion proteins (Tpr, Nup98, Nup214). In each case we consider the normal function of the nucleoporin and its translocation partners, as well as what is known about their mechanistic contributions to carcinogenesis, particularly in leukemias. Studies of nucleoporin-linked cancers have revealed novel mechanisms of oncogenesis and in the future, should continue to expand our understanding of cancer biology.

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Abbreviations:
NPC, nuclear pore complex; cNLS, classical nuclear localization signal; NES, nuclear export signal; FG, phenylalanine-glycine; FxFG, phenylalanine-any amino acid-phenylalanine-glycine; GLFG, glycine-leucine-phenylalanine-glycine; AA, amino acid.

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1. The nuclear pore complex

The nuclear pore complex (NPC) is a massive, multiprotein structure responsible for traffic between the nucleus and cytoplasm. The general structure of the NPC is becoming fairly well defined, but the mechanism of translocation through the pore remains incompletely understood. The NPC is comprised of a central core region consisting of nucleoplasmic and cytoplasmic rings joined by a spoke structure (Fig. 1). These are anchored in the nuclear membrane through three
transmembrane proteins. Cytoplasmic filaments and the nuclear basket of the pore extend out from this central structure. Pore proteins or nucleoporins (designated Nup followed by their predicted molecular weight) are modular in that a limited number of structural motifs (coiled-coils, α-solenoids, β-propellers) are used repeatedly to build the symmetrical NPC structure. Approximately one-third of nucleoporins contain a domain of phenylalanine-glycine (FG) motifs interspersed with spacer sequences. These repeat domains are natively unstructured and serve as interaction sites for transport receptors (karyopherins) that escort cargo through the pore. For more information on NPC structure and function, a number of excellent reviews are available [1–5].

2. Nup88: a potential marker for high grade tumors

2.1. Nup88 in multiple subcomplexes of the NPC

Nup88 is a non-FG nucleoporin found exclusively on the cytoplasmic face of the NPC (Fig. 1). This nucleoporin is comprised of two of the repeating structural motifs of the pore; the N-terminal domain is predicted to form a β-propeller structure and the C-terminal domain contains predicted coiled-coils (Fig. 2A). Nup88 is found in a biochemically defined subcomplex of the pore together with the FG repeat nucleoporin Nup214 [6,7] and, in some systems, Nup62 [8]. Formation of this complex is mediated through the N-terminal β-propeller of Nup88 and a central coiled-coil domain of Nup214. Findings differ on whether Nup88 directs Nup214 to the NPC or whether both components of the complex are required simultaneously for NPC association. Approximately one-third of nucleoporins contain a domain of phenylalanine-glycine (FG) motifs interspersed with spacer sequences. These repeat domains are natively unstructured and serve as interaction sites for transport receptors (karyopherins) that escort cargo through the pore. For more information on NPC structure and function, a number of excellent reviews are available [1–5].

2.2. The Nup214/Nup88 subcomplex in nuclear transport

The Nup214/Nup88 subcomplex does not seem to be directly required for nuclear import, but it makes important, if somewhat controversial, contributions to nuclear export. Both the FG repeat domain of Nup214 and the N-terminal β-propeller domain of Nup88 have been reported to bind directly to CRM-1/exportin-1, the receptor for export of most proteins from the nucleus [7,12]. The Nup214/Nup88 subcomplex is crucial for CRM-mediated export of the 60S ribosomal particle from the nucleus although, unexpectedly, this required the Nup88-binding coiled-coils of Nup214 but not the Nup214 FG domain [13]. However, the contribution of the Nup214/Nup88 complex in the export of nuclear export signal (NES)-containing proteins varies between systems and substrates. In HeLa cells, depletion of the Nup214/Nup88 complex leads to greatly diminished export of the nuclear transcription factor NFAT [14] but has a minimal effect on export of a classical nuclear localization sequence (cNLS)-GFP-NES reporter [13]. In cultured Drosophila S2 cells, depletion of either Nup214 or Nup88 did not alter the localization of a GFP-NES reporter [15]. In contrast, Drosophila larvae lacking either Nup214 or Nup88 show a striking cytoplasmic accumulation of the NF-κB homologs, Dorsal and Dif, which contain NESs and normally shuttle between nucleus and cytoplasm. These results led the authors to propose that the normal function of Nup214/Nup88 in larvae is to retain CRM at the NPC and attenuate rather than enhance nuclear export [9]. The variation in reported roles for Nup214 and Nup88 in nuclear protein export may reflect either a degree of redundancy in cultured cells that is lacking in embryos, or differences in the specific requirements of individual protein cargoes used in the assays.

A surprising finding came from studies in Drosophila demonstrating that Nup88 (known in D. melanogaster as members only,
Fig. 2. Nucleoporin structure and rearrangement following chromosomal translocations. The four nucleoporins discussed are depicted with major structural elements, along with their translocation partners, if applicable, and the structure of the resulting chimeric fusion proteins. Abbreviations: β-prop, β-propeller; c-c, coiled-coil; RTK, receptor tyrosine kinase; P/S, proline/serine rich; TM, transmembrane; JM, juxtamembrane; HD, homeodomain; BD, binding domain; Ub, ubiquitination; P, phosphorylation.

Table 1

<table>
<thead>
<tr>
<th>Mammals</th>
<th>S. cerevisae</th>
<th>S. pombe</th>
<th>C. elegans</th>
<th>D. melanogaster</th>
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<tbody>
<tr>
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<td>–</td>
<td>–</td>
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<tr>
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<td>Nup145N, Nup100, Nup116</td>
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</tr>
<tr>
<td>Nup214, CAN</td>
<td>Nup159</td>
<td>Nup146</td>
<td>Nup146</td>
<td>Nup214</td>
</tr>
</tbody>
</table>

mb; Table 1) was expressed in a tissue-specific manner [16]. Although some other Nups exhibit tissue-specific expression [17–19], given the role of Nup88 in directing other nucleoporins to the NPC, it was not expected that it would be absent from many developing tissues. In Nup88 null larvae, NF-κB homologs did not relocalize to the nucleus of fat body cells in response to immune stimuli, perhaps due to increased nuclear export in the absence of Nup88. However, in tissues where Nup88 was not normally not expressed, NF-κB homologs were correctly relocalized, suggesting that Nup88 is not universally required for regulation of nuclear export. Presumably there are redundant pathways that function in tissues lacking Nup88.

2.3. Nup88 overexpression in tumor tissues

The association of Nup88 with tumors first developed when a monoclonal raised against Candida albicans proved to cross react with a variety of tumor cells and was then found to recognize Nup88 [20]. An antibody was then raised specifically against Nup88 and immunohistochemistry revealed overexpression of Nup88 in 75% of ovarian tumors [21]. This analysis was extended to a variety of tumor types and Nup88 was consistently found to be overexpressed in a broad spectrum of neoplasias. Staining was especially evident in carcinomas but was also observed in sarcomas, lymphomas and mesotheliomas [22,23]. Immunoblotting of several lung carcinoma samples suggested that overexpression of Nup88 does not correlate with overexpression of the other nucleoporins tested, Nup214 and Nup153 [22]. PCR quantitation of Nup88 and Nup107 mRNAs derived from tumors indicated that the level of Nup88, but not Nup107, transcripts correlated with a malignant phenotype [24]. Thus, overexpression seems to be unique to Nup88 rather than the result of increased production of NPCs or upregulation of the Nup214/Nup88 subcomplex. In tumors, Nup88 staining is prominent in the cytoplasm, often in granular dots. This correlates well with earlier studies indicating that, when transiently overexpressed in cultured cells, Nup88 accumulates in the cytoplasm [6]. Interestingly, in normal fetal tissue samples, strong Nup88 staining was observed in only a subset of tissues, including developing lung epithelia and intestinal crypts [23].

In tumor tissues, the intensity of Nup88 staining has been noted to correlate with tumor grade. Benign tumors and mild hyperplasias showed little to no evidence of overexpression, whereas Nup88 overexpression was routinely detected in more advanced tumors. Highest expression was frequently noted around the edges of tumors suggesting a link to invasivity [22]. High Nup88 expression was correlated with tumor aggressiveness in breast, colorectal and hepatocellular carcinomas [24–26]. Nup88 expression seems to increase over the progression of carcinogenesis and to be most associated with poorly differentiated tumors. For these reasons, Nup88 has been proposed as a marker of tumor state and a potential indicator of patient prognosis.

The exact nature of the link between Nup88 expression and cancer is uncertain. In Drosophila, active NF-κB accumulates in the cytoplasm due to its increased nuclear export in the absence of Nup88. Conversely, it may be that overexpression of Nup88 in cancer cells leads to decreased NF-κB export, resulting in accumulation in the nucleus. Indeed, in many human tumor types including breast, pancreas and colon carcinomas, NF-κB is predominantly found in the nucleus where it could contribute to constitutive upregulation of target genes [27]. Possibly there are additional sig-
naling molecules that also accumulate in the nucleus and alter transcription patterns following overexpression of Nup88, but such a potential mechanism remains to be thoroughly investigated.

3. Tpr: an activator of receptor tyrosine kinases

3.1. Tpr structure and normal function

Tpr is a 265 kDa nucleoporin found exclusively at the nucleoplasmic face of the pore where it is a major component of the nuclear basket (Fig. 1) [28]. Tpr does not possess nucleoporin FG repeats but it does contain numerous heptad repeat or leucine zipper motifs (Fig. 2B). Tpr is comprised of two domains, a large, approximately 190 kDa N-terminal domain which contains the heptad repeats and an unstructured, acidic C-terminal domain [29]. The heptad repeat region is predicted to form a coiled-coil structure and indeed Tpr has been characterized as an extended, rod-like homodimer in which the coiled-coils mediate dimerization [30]. The C-terminal domain contains an NLS responsible for import of Tpr to the nucleoplasm, whereas the region required for assembly into the NPC is found within the N-terminal coiled-coil domain.

At the NPC, Tpr is a major component of the nuclear basket (Fig. 1) where it binds to Nup153; when Nup153 is depleted using siRNA, Tpr is released from the NPC into the nucleoplasm. Tpr has also been reported to form filamentous structures that emanate from the nuclear basket and extend into the nucleolar interior of amphibian oocytes [31] although the presence of such filaments in cultured somatic cells is controversial [32,33]. Functional roles for Tpr in both protein and mRNA export have been suggested. Most recently, Tpr has been reported to function in the mitotic spindle checkpoint as a binding partner and regulator of Mad1 and Mad2 [34].

Tpr orthologs have been studied in Drosophila, where it is also known as Megator, and in yeast, where two orthologs have been identified, Mlp1 and Mlp2 (Table 1). The double Mlp1/2 null mutant in yeast remains viable although with increased sensitivity to DNA damage [35]. In contrast, deletion of Megator is embryonic lethal in flies. Drosophila Tpr/Megator is found both at the NPC and in the extra chromosomal spaces of the interphase nucleolus [36]. During mitosis, Megator is reported to colocalize with a spindle matrix structure through association with the Skeletor and Chromator proteins [37]. The yeast Mlp1/2 proteins are found at the NPC and at distances into the nucleolar interior although it is not certain whether they form into filamentous structures [35], Mlp1/2 have been reported to play multiple roles in functions including protein export, and quality control/export of mRNA [38,39]. Further, Mlp1 and 2 are thought to anchor a variety of functional machinery at the NPC including transcriptional co-activators, some activated genes [40] and, paradoxically, silenced telomeres [41].

3.2. Tpr translocations lead to dimerization of Met and NTrk1

In contrast to the typical Nup nomenclature of nucleoporins, Tpr was named for its initial isolation from a carcinogen-treated osteogenic sarcoma line as part of a chromosomal translocation (1q25:7q31) that fused N-terminal sequences of Tpr (translocated promoter region) to the kinase domain of the proto-oncogene, Met [42]. The resulting 65 kDa fusion protein combines two heptad repeats of Tpr with the tyrosine kinase domain of Met (Fig. 2B). Dimerization through the heptad repeats leads to constitutive activation of Met kinase activity independent of ligand binding [43]. Tpr/Met was the first such activating fusion of a tyrosine kinase receptor to be identified and, served as the prototype for understanding the now more than 25 oncogenes in which translocated tyrosine kinase receptors are activated by dimerization [44].

Met is the cell surface tyrosine kinase receptor for hepatocyte growth factor (HGF), also known as scatter factor (reviewed in [44]). Both Met and HGF have essential roles in cell migration during development, as well as in repair of adult tissues and in angiogenesis. In the chromosomal translocation, the extracellular ligand binding and transmembrane domains of Met are lost and the resulting cytosolic fusion protein is no longer subject to downregulation by endocytosis. Also missing is the juxtamelane domain, which normally functions in downregulation of activated receptor through ubiquitin-mediated recruitment of the proteolytic machinery to internalized receptor [44]. Thus, multiple checks on the Met kinase are lost and the cytosolic Tpr/Met fusion protein becomes a constitutive activator of Ras/MAPK and PI3K pathways. Tpr translocations are infrequent in human tumors, but Tpr-Met translocation is associated with gastric carcinomas where it is thought to represent an early step in carcinogenesis [45,46].

Tpr has also been found translocated with NTrk1 (TrkA), the transmembrane tyrosine kinase receptor for nerve growth factor (reviewed in [47]). Tpr/NTrk1 fusion proteins are very similar in structure to the Tpr/Met rearrangements, juxtaposing either 2 or 10 heptad repeats with the NTrk1 kinase domain (Fig. 2B). Interestingly, the NPC-targeting site of Tpr is present within the larger of these fuses, although association of the fusion protein with the NPC has not been shown. As in Tpr/Met, the Tpr/NTrk1 translocation leads to constitutively active, unregulated NTrk1 signaling. Tpr-NTrk1 is one of several translocations of the NTrk1 receptor that are associated with papillary thyroid carcinoma, the most common type of thyroid cancer.

4. Nup98: an aberrant regulator of transcription

4.1. Nup98 structure, variants and binding partners

The FG-repeat nucleoporin Nup98 is the only vertebrate nucleoporin with substantial numbers of GLFG-type repeats [48,49]. Nup98 is expressed in two major forms that vary as a result of differential splicing. The first splice variant encodes a 920 amino acid protein (Fig. 2C); the N-terminal half contains FG and GLFG repeat motifs and is bisected by a small coiled-coil domain (AA 181–224) that binds to the β-propeller nucleoporin Rae1/Gle2 (hereafter referred to as Rae1) [50]. Rae1 is implicated in mRNA export, functions in a cell cycle checkpoint, and, as part of a distinct, RNA-containing complex, has a role in mitotic spindle function [50–52]. At the C-terminus of Nup98 is a domain with a unique, β-sandwich structure [53] which possesses autoproteolytic activity [54] and is also important for directing Nup98 to the nuclear pore complex [11,55]. The second major splice variant encodes a 186 kDa Nup98–Nup96 polyprotein which is identical to Nup98 in its first 915 amino acids and goes on to encode a second, non-FG repeat nucleoporin, Nup96. The autoproteolytic activity of Nup98 cleaves both variants after amino acid 863 to produce the 90 kDa mature protein known as Nup98 and either an 8 kDa tail of no known function, or the mature Nup96 nucleoporin [54]. Since all chromosomal translocations occur within the Nup98 coding region (see Section 4.3), Nup98 sequences never appear in translocation fusion proteins and will not be further discussed here.

Nup98 and Rae1 comprise a distinct subcomplex of the NPC (Fig. 1). Both are dynamic components of the NPC, meaning that they can move on and off the pore, although their dissociation from the NPC is not as rapid as the most dynamic nucleoporins Nup153 and Nup50 [56,57]. Nup98 can also be found within the nucleolus, where it is often dynamically associated with intranuclear bodies termed GLFG bodies for the domain required for association with these structures. Additionally, Nup98 can shuttle between nuclear and cytoplasmic compartments. It is not yet known whether Nup98 plays a functional role within the nucle-
oplasm, but its mobility within that compartment is blocked by inhibitors of transcription [56]. Nup98 is found on both nuclear and cytoplasmic faces of the NPC through association of the C-terminal domain with the structural Nup107 complex (Fig. 1) [53,55]. The same region of Nup98 also binds to Nup88 and these two interactions appear to be mutually exclusive. Although the C-terminal domain of Nup98 has specific nucleoporin binding partners, it alone is not efficient at directing Nup98 to the NPC but rather requires additional interactions mediated by the GLFG repeats to stabilize it at the NPC [11].

4.2. Nup98 function in transport, viral infection and mitosis

Nup98 has been implicated in a variety of activities and the functional contribution of this nucleoporin is emphasized by the finding that the Nup98 knockout mouse dies early in embryonic development [58]. The GLFG repeat region of Nup98 binds transport receptors including importin β, transportin, and the mRNA export receptor TAP [32,49,59]. Nuclei assembled in the absence of Nup98 are competent for cNLS-mediated nuclear import, but show structural and replication defects in keeping with a possible role for Nup98 in other import pathways or in NPC assembly [48]. Indeed, in cells from the Nup98 knockout mouse, several other nucleoporins were displaced from the NPC in the absence of Nup98 [58]. Injection of Nup98 antibodies into oocyte nuclei blocked all RNA export from the nucleus with the exception of tRNA [60]. The involvement of Nup98 in mRNA export is supported by exciting work from viral systems wherein components of host pathways are often targeted or redirected in order to favor viral replication. The vesicular stomatitis virus protein M binds the Nup98/Rae1 subcomplex through the Rae1 protein and thus blocks cellular mRNA export [61]. Both influenza and polio viruses target Nup98 for degradation early during infection [62,63]. Conversely, IFN-γ, which is induced in cells to combat viral infection, activates transcription of both the Nup98 and Rae1 genes in order to compensate for these viral attacks [64].

Mitotic roles for several nucleoporins have been characterized recently and Nup98 also figures prominently at this stage in the cell cycle. The Nup98/Rae1 complex binds to the cdh1-form of the anaphase promoting complex/cyclosome (APC/C) [65]. The APC/C targets securin, the inhibitor of the separase enzyme, for degradation at the metaphase to anaphase transition. Degradation of securin allows separase to cleave the cohesin complex and permit sister chromatid separation. The cdcc20 form of the APC/C has been considered responsible for securin targeting, however reduction in Nup98/Rae1 levels led to increased securin stability, thus implicating the cdh1-APC/C in this process as well. The functional significance of interaction between Nup98/Rae1 and the APC/C is underscored by the observation of aneuploidy in 32% of splenocytes from Nup98+/-/ Rae1+/- double heterozygous mice [66]. In an apparently distinct mitotic role, Rae1 was found in a large complex of proteins and RNA that is required for mitotic spindle assembly, although the presence of Nup98 in this complex was not directly demonstrated [52]. Additionally, the region of Nup98 between the repeats and the autoproteolytic domain has recently been found to play a role in the regulation of microtubule dynamics during spindle assembly [151].

4.3. Nup98 translocation partners: homeodomains and more

The first indication of a role for Nup98 in cancer emerged when two groups simultaneously reported Nup98 as part of a rare but recurrent chromosomal translocation in AML patients [67,68]. This translocation (7p15:11p15) fuses the 5′ half of the NUP98 gene with the 3′ portion of the HOXA9 gene (Fig. 2C). The resulting 59 kDa fusion protein is expressed under control of the Nup98 promoter and contains nearly all the FG/GLFG repeats of Nup98 together with the Rae1 binding site, and the DNA-binding homeodomain of

### Table 2

<table>
<thead>
<tr>
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<th>Partner function</th>
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AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; CML: chronic myeloid leukemia; T-ALL: T-cell acute lymphoblastic leukemia; ABL: acute biphenotypic leukemia; t-MDS: therapy-related myelodysplastic syndrome; AUL: acute undifferentiated leukemia; NGF: nerve growth factor.
HoxA9. The Nup98/HoxA9 fusion protein is not found at the NPC but rather is localized to the nucleoplasm in the form of finely punctate foci [69]. Given this localization it seems likely that the nuclear transport function of Nup98 is not compromised by the fusion protein.

Homeodomain proteins (encoded by the homeobox genes) are transcription factors that function as master regulators of development and differentiation [reviewed in [70]]. The human genome contains 4 clusters (HOXA–D) of class I homeobox genes that arose from duplications of a single ancestral cluster in *Drosophila*. Individual clusters contain a representative of 8–11 out of the 13 paralogs from duplications of a single ancestral cluster in *Drosophila*. Many other, less well conserved, class II homeobox genes are scattered throughout the genome. The class II-encoded proteins, Pbx1 and Meis1, act as Hox co-factors and work in concert with Hox transcription factors during hematopoiesis (Pbx1 works with paralogs 1–9, Meis1 works with paralogs 9–13). Independently, the Hox proteins bind DNA with relatively little selectivity and interaction with these cofactors increases both their affinity and specificity for DNA.

Subsequent to identification of the Nup98/HoxA9 translocation, Nup98 has been found in chromosomal translocations with a remarkably wide array of partners (Table 2). In all cases, the fusion proteins carry the FG/GLFG domains of Nup98, typically the first 469 amino acids as are present in Nup98/HoxA9. Many of these Nup98 fusion partners are homeodomain transcription factors from both class I and class II. Class I Nup98 partners come from the A, C and D clusters and represent genes from the more distal paralogs of each cluster. Nup98/homeodomain translocations are most often found in AML patients but also have been associated with chronic myeloid leukemia, as well as the pre-leukemic myelodysplastic syndrome (MDS).

Non-homeodomain fusions have been identified in AML, MDS and, albeit infrequently, in other forms of leukemia as well (Table 2). The non-homeodomain Nup98 partners span a wide variety of proteins, most with nuclear functions. These include topoisomerases I and IIb, histone modifying enzymes (NSD1 and NSD3 methyltransferases, and the jumonji domain demethylase, Jarid1A), and the transcriptional co-activator LEDGF. Interestingly, the Nup98 partner protein, SetBp1, interacts with Set, a partner in a Nup214 chromosomal translocation (see Section 5.2). Taken together, these as well as the Hox partners strongly implicate alteration of chromatin structure and resulting changes in transcription as a mechanism for Nup98-induced leukemogenesis. However, other partner genes, encode an RNA helicase, an actin-binding complex subunit, a guanine nucleotide exchange factor, and several proteins of unknown function which raise the possibility of additional mechanistic contributions to leukemogenesis.

4.4. Nup98 fusions and aberrant transcriptional regulation

A major advance in understanding Nup98 leukemias came with the work of Kasper and colleagues [69] who demonstrated that the FG/GLFG repeat domain of Nup98, when directed to DNA, activates transcription through recruitment of the co-activator CBP/p300, a histone acetyltransferase. This finding strongly implicated altered transcriptional regulation in the development of Nup98 leukemias (Fig. 3). Interestingly, this same domain of Nup98 was found to couple the mobility of Nup98 within the nucleus to ongoing transcription, although the basis for this remains unclear [56]. Others have subsequently shown that Nup98/PMX, a class II gene fusion protein, can bind both CBP/p300 and HDAC [71].

Microarray studies of Nup98/HoxA9-expressing, CD34+, peripheral blood progenitor cells confirmed that a number of genes are indeed upregulated in these cells, among them HOXA5, A6, A7, and A9, as well as the gene encoding the Meis1 cofactor. A much smaller number of genes are downregulated but among these are genes encoding CCAAT/enhancer binding proteins (C/EBP α and ε), which promote myeloid cell differentiation [72,73]. Cells expressing Nup98/HoxD13, Nup98/PMX1, Nup98/HHEX or the artificial Nup98/HoxA10 fusion, all showed similar gene expression profiles [74–76].

4.5. Mouse models of Nup98 leukemogenesis

Overexpression of endogenous HoxA9 and Meis1 is frequently observed in AML patients [77] where overexpression of HoxA9 is correlated with poor prognosis [78]. HoxA9 overexpression in mouse bone marrow transplantation (BMT) models (bone marrow cells transduced with retroviral vectors driving HoxA9 expression are transplanted into mice), leads to leukemia in approximately 4 months but when combined with overexpression of Meis, the time required for progression to leukemia is halved [79]. In the mouse BMT model, Nup98/HoxA9, Nup98/HoxD13, or Nup98/PMX1 expression resulted in myeloproliferative disease and, in many reports, progression to AML after latency periods approaching a year (summarized in [77]). This long latency suggests that, while Nup98 fusions increase proliferation and attenuate differentiation of hematopoietic progenitors, accumulation of additional genetic perturbations is required for full progression to leukemia.

As determined for HoxA9, overexpression of Meis1 can cooperate with the Nup98/homeodomain fusions to facilitate leukemogenesis in mouse models [75,79,80]. Upregulation of the *MEIS1* gene is thus a potential “second hit” although the mechanism by which Meis1 works with Nup98/HoxA9 remains uncertain as the fusion protein does not retain the ability to bind Meis1. Nup98/HoxA9 expression can immortalize HoxA9−/− cells in vitro, suggesting that HoxA9 is not absolutely required [81]. Thus, elevated levels of Meis1 may work with other Hox factors to aberrantly activate additional pathways. Interestingly, the gene encoding Flt3, a receptor tyrosine kinase, is a target of Meis1 transcriptional regulation and elevated Flt3 levels have been observed in AML patients [77]. Overexpression of Flt3 can stimulate progression to AML by Nup98 fusions [82]. Although not normally expressed in Nup98 AML patients, both the Bcr/Abi and Tel/PDFG activated tyrosine kinases can also cooperate with Nup98/homeodomain fusions [83,84].
Methyltransferase activity, caused upregulation of both
fusion of Nup98 with a non-HD partner possessing histone
transcription by Topoisomerase I[86,87]. Consistent with this, catalytic activity
was not essential for the previously reported coactivation of
in a BMT mouse model[85]. Consistent with this, catalytic activity
was not required for leukemogenesis by the Nup98/TopoI fusion
consensus sequence binding protein) does accelerate leukemogen-
éssis by Nup98/TopoI. Interestingly, topoisomerase catalytic activity
was not reported in mouse BMT
models, but deficiency of the tumor suppressor gene, IC SBP (IFN
consensus sequence binding protein) does accelerate leukemogen-
esis by Nup98/TopoI. Interestingly, topoisomerase catalytic activity
was not required for leukemogenesis by the Nup98/TopoI fusion
in a BMT mouse model [85]. Consistent with this, catalytic activity
was not essential for the previously reported coactivation of
transcription by Topoisomerase I[86,87].

Recently, Wang and colleagues established that Nup98/NSD1,
a fusion of Nup98 with a non-HD partner possessing histone
methyltransferase activity, caused upregulation of both HOXA9 and
MEIS1 genes. Activation of transcription correlated with binding of
the fusion protein to the co-activator CBP/p300 [88]. Simi-
larly, Nup98/IQCG binds to both CBP/p300 and HDAC [89], and
Nup98/PMX1 and Nup98/HoxD13 specifically activate the HoxA9
promoter [75]. Thus upregulation of HoxA9 and Meis expression
may provide a unifying mechanism to link leukemogenesis induced
by members of both classes of Nup98 fusions. However, the means
by which Nup98 fusion proteins recognize specific promoters is
uncertain and it remains to be seen whether additional non-HD
fusions will similarly activate these genes.


5.1. Nup214 structure and function in the NPC

Nup214 is an FG repeat nucleoporin normally confined to the
cytoplasmic face of the NPC (Fig. 1). Nup214 contains repeats of both
the FG and ExFG type and its repeat domain is a high affinity binding
site for the nuclear protein export receptor, CRM-1/exportin-1.
The N-terminus of Nup214 is predicted to form a β-propeller struc-
ture, as has been demonstrated for the yeast ortholog, Nup159 [90].
This is followed by a region rich in proline and serine (17% and 25%,
respectively), two stretches of predicted coiled-coil structure, and
a structurally uncharacterized region (Fig. 2D). The nucleoporin FG
repeats are found at the C-terminus of Nup214. As detailed in Sec-
tions 2.1 and 2.2, Nup214 is found in a subcomplex of the NPC,
together with Nup88. Several studies point to a role for Nup214
in mRNA export and indeed, the S. cerevisiae Nup214 homolog,
Nup159 (Table 1), recruits the RNA helicase Dpb5, a co-factor in
mRNA export [90,91]. Nup214 is essential during development; Nup214−/−
embryos died once maternal stores of Nup214 were
depleted and were impaired in both protein import and mRNA
export [92]. In contrast, nuclei reconstituted from Xenopus egg
extracts depleted of Nup214 showed little to no defect in nuclear
protein import [93]. Since nuclear export is not monitored in the
Xenopus system, it may well be that the critical contribution of
Nup214 is in nuclear export.

Nup214 is most frequently described as a component of cyto-
plasmic fibrils of the pore, however Xenopus nuclei lacking Nup214
appeared by EM to have intact fibrils that were lost in the absence
of Nup358 [93]. Alternatively, Nup214 has been proposed to form
shorter, more central fibers or to be associated with the NPC cyto-
plasmic ring. An intriguing study using antibodies to individual
domains of Nup214 found that whereas the N-terminus remains
anchored on the cytoplasmic face, the C-terminal repeat domain is
untethered and can reach through the center of the pore, possibly
as far as the nuclear basket [94].

5.2. Nup214 fusion with Dek and Set chromatin binding proteins

Like Tpr, the Nup214 gene was first identified as a component of
a chromosomal translocation and, presumably due to its proxim-
ity to the c-Abi gene, was named Cain (CAN). The protein came to
be referred to as Can but has since transitioned to the more stan-
dard nomenclature of Nup214. This first chromosomal translocation
(6p23:9q34) joined the DEK and CAN genes [95] and was followed
shortly thereafter by identification of an intrachromosomal translo-
cation (9q32:9q34) that fused the SET and CAN genes [96]. These
translocations result in expression of fusion proteins that join virtu-
ally the full Dek or Set protein to the C-terminal two-thirds of
Nup214, including a portion of the coiled coil domain and the FG
repeat domain (Fig. 2D).

Dek and Set are abundant nuclear phosphoproteins of 45 and
39 kDa respectively, each with a significant stretch of acidic amino
acids. Both are major components of chromatin and are thought to
influence transcription through effects on chromatin modification.
Set was identified as a member of the INHAT complex (inhibitor
of histone acetylation and transcriptional activation) [97]. How-
ever, paradoxically, Set was found at transcriptionally active sites
in Drosophila polytene chromosomes and identified as an activa-
tor of transcription from chromatin templates in vitro [98,99]. Set
(also known as TAF-1β) was also identified as an inhibitor of protein
phosphatase 2A [100].

Dek binds to both naked DNA and chromatin, and, like INHAT,
has been described as an inhibitor of the histone acetyltransferase
activity of both p300/CBP and PCAF [101]. Dek was also identified
as a co-repressor of NF-κB-dependent transcription [102]. Inter-
estingly, Dek itself is acetylated and this modification appears to drive
relocalization of Dek from chromatin to speckles/interchromatin
granule clusters [103]. Phosphorylation similarly releases Dek from
chromatin [104] and phosphorylated Dek can bind to the splicing
factor U2AF35 and increase the specificity of 3′ splice site recogni-
tion [105]. Srm160, a splicing coactivator has also been reported
to interact with Dek [106]. In that study, Dek remained associated
with the spliced RNA, which raises the possibility that Dek might
do influence post-splicing events such as proofreading or cou-
ing to the mRNA export system. Dek has also been implicated in
protection from p53-dependent apoptosis [107].

An intriguing mechanism by which Dek and Set act as opposing
factors to regulate chromatin modification and accessibility to
transcription initiation factors has been proposed [108]. Addition of
Set releases both Dek and PARP from chromatin templates in
vitro and leaves the chromatin more accessible for transcriptional
activation. Subsequently there may be a second contribution from
Set in recruitment of the Mediator co-activating complex to the
chromatin.

The Dek/Nup214 fusion was identified in a subtype of AML char-
acterized by early age of onset and generally poor response to
therapy [109]. Set/Nup214 was reported in one patient with acute
undifferentiated leukemia (AUL) [96]. In a few patients with T-cell
acute lymphoblastic leukemia (T-ALL), the same SET/CAN fusion
was generated by deletion of the intervening region of chromosome 9
[110,111].

Given the many reports of Dek and Set as modifiers of chromatin
structure, a reasonable expectation is that, like Nup98 fusions,
Nup214 fusions might similarly alter transcription patterns during
hematopoiesis. Surprisingly, there are relatively few mechanistic
studies of leukemogenesis by the Dek/Nup214 or Set/Nup214 fusion
proteins. Early on, Fornerod et al. observed that Dek/Nup214 or
Set/Nup214 fusion proteins are localized in a punctate fashion in
the nucleoplasm and no longer associate with the NPC [112]. Some
binding of the fusion protein to Nup88 may persist, despite the loss
of half of the coiled-coil region involved in Nup88 binding (Fig. 2D)
[113]. Nup214 fusion proteins do retain the ability to bind to and
relocalize Crm-1, the NES receptor, raising the possibility of altered
nuclear transport in leukemic cells [113,114].

Set was found to be overexpressed in cells from a variety of solid
 tumors including uterine, stomach and rectal tumors [115]. How-
ever, neither fusion protein increased proliferation of a myeloid
precursor cell line, although Set/Nup214 did block differentiation of
these cells [110,116]. Following BMT of Set/Nup214 express-
ing cells into mice, expansion of an early progenitor cell pool and reduction in peripheral lymphocytes were observed, but the mice showed no tendency to develop leukemia [115]. ChiP experiments with Set/Nup214-expressing T-ALL patient cells indicated that Set/Nup214 bound to a subset of HoxA gene promoters. HoxA expression is normally seen in only the earliest T-cell precursors, thus expression of these genes could enforce a block in T-cell maturation [111]. It may be that the role of Set/Nup214 in leukemogenesis is limited to this block to differentiation.

Like Set, Dek overexpression has been reported in solid tumors including hepatocellular carcinoma, glioblastoma and melanoma, as well as in AML [117]. Surprisingly, Dek/Nup214 was found to activate global protein translation only in myeloid-derived cell lines, a property that required both Dek and Nup214 sequences and thus may be specific to the fusion protein [118]. Whether the contribution of the Dek/Nup214 and Set/Nup214 fusions to leukemogenesis is simply through blocks to differentiation or whether any of the diverse functions ascribed to Dek and Set will play a significant role remains to be seen.

5.3. Nup214 translocation activates abl kinase activity

A third and unique Nup214 translocation was identified in a screen for novel Abl translocations associated with T-cell ALL [119]. Approximately 6% of T-ALL patients examined exhibited a marked, extrachromosomal amplification of the ABL1 gene. This resulted from amplification of an episome derived from circularization of a small region of chromosome region 9q34. The episome contains the 3′ half of the ABL1 gene encoding the Abi kinase, the entire LAMC3 gene, which encodes a protein found on the apical surface of ciliated epithelial cells, and the majority of the CAN gene. Circularization generates an in-frame fusion of the CAN and ABL gene fragments and leads to expression of a Nup214/Abi fusion protein (Fig. 2D). Analogous to BCR/ABL translocations, the breakpoint in the ABL1 gene is always within the first intron, leading to inclusion of exon 2 which encodes most of the Abi protein including the SH3, SH2, and tyrosine kinase domains. Nup214 contributes its β-propeller, P/S-rich, and coiled-coil domains as well as a variable amount of the FG repeat domain. In one patient, the Nup214 breakpoint occurred just downstream of the coiled-coil domain suggesting that the nucleoporin FG repeats may not be essential for leukemogenesis.

The Nup214/Abi protein is an active tyrosine kinase [119], but the means of constitutive kinase activation was not readily apparent until a recent study characterized a novel potential mechanism for activation of a tyrosine kinase [120]. The Nup214/Abi fusion protein retains the ability to associate with the NPC through binding of the Nup214 coiled-coils to Nup88. Since Nup214/Abi is significantly overexpressed from multicopy episomes (5–50 copies per cell [119], it may compete effectively with endogenous Nup214 for sites at the NPC). The resulting close association of Nup214/Abi proteins at the NPC would allow for some activation of kinase activity, leading to phosphorylation and constitutive activation of adjacent fusion proteins. This model is supported by experiments in which constructs encoding the Nup214 coiled-coil domain compete for Nup214/Abi association with the NPC and for downstream indicators of kinase activity. A direct test of this mechanism of kinase activation would be the ability of a minimal Nup214 (coiled-coil domain)/Abi fusion to drive kinase activation and induce T-ALL in mice.

Given the cytoplasmic orientation of Nup214 at the NPC, it is possible that Nup214/Abi would be in sufficient contact with the cytoplasmic substrates to allow for activation of signaling. Alternatively, the Nup214/Abi kinase may be ideally positioned to contact substrates as they translocate through the NPC. Nup214/Abi phosphorylates other nucleoporins such as Nup358, although whether this plays a part in oncogenic transformation is unknown. Nup214/Abi is a relatively weak oncogenic protein, requiring 70–200 days after BMT of cells expressing Nup214/Abi to induce T-cell disease in mice. This is compared to the short 20 days latency of Bcr/Abi-induced myeloproliferative disorder in the same system [120].

6. Conclusions

The study of nucleoporins and their roles in cancer has been a fruitful one, revealing novel mechanisms by which cells are advanced along the path to transformation. From our current level of understanding, each nucleoporin contributing to carcinogenesis seems to do so in a unique manner. It is somewhat surprising that, in the case of nucleoporin translocations, defects in nuclear transport have not been reported. Patient cells are heterozygous for these translocations and thus the level of the nucleoporin involved should be halved. This has not been thoroughly investigated, but perhaps the NPC is remarkably able to compensate for reduction in the level of many of its components thus circumventing selection against nucleoporin-based transformation. A recent report indicated that knockout of Nup358 also leads to increased aneuploidy in mice [121] although this nucleoporin has not yet been implicated in any form of cancer. It may be that additional Nups will prove to contribute to cancer in novel ways and the ongoing study of nucleoporin-induced cancers may continue to uncover surprising new mechanisms in cancer biology.

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