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NUP98 and *KMT2A*: usually the bride rather than the bridesmaid

Alexandre Fagnan[1,2,3] and Thomas Mercher[1,2,3,4]

[1]INSERM U1170, Gustave Roussy Institute, Villejuif; [2]Université Paris Diderot, Paris; [3]Equipe labellisée Ligue Nationale Contre le Cancer, Paris and [4]Université Paris-Saclay, Villejuif, France

E-mail: THOMAS MERCHER - thomas.mercher@inserm.fr

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In human hematopoietic malignancies, *KMT2A* and *NUP98* are each independently targeted by numerous chromosomal alterations leading to the expression of fusion oncogenes. In this issue of *Haematologica*, Fisher and colleagues from J. Schwaller's team report the functional study and creation of an *in vivo* model[1] for a unique fusion between these two genes[2] showing that leukemia development by NUP98-KMT2A is not associated with classical KMT2A fusion mechanisms.

KMT2A (a.k.a. MLL) is a large protein of almost 4,000 amino acids that is processed by the endopeptidase Taspase1. It interacts with numerous proteins and assembles into large protein complexes (Figure 1). The functions of KMT2A include writing the H3K4me3 chromatin mark characteristic of active promoter regions through its C-terminal SET domain. In both lymphoid and myeloid malignancies, KMT2A is targeted by numerous chromosomal alterations resulting in the expression of fusion oncogenes with over 80 different partners *in toto* (*https://mitelmandatabase.isb-cgc.org/*). Experimental models have demonstrated that several fusions containing the N-terminal portion of KMT2A and various partners [here termed KMT2A-X, where X is frequently AFF1, MLLT3, MLLT10 or MLLT1 in acute lymphoid leukemia patients, and MLLT3, MLLT10, MLLT1 or ELL in patients with acute myeloid leukemia (AML)] are important for disease development and maintenance.[3,4]

It has long been recognized that KMT2A-X fusions activate transcription of different *HOX* genes (e.g. *HOXC8*, *HOXA7*, *HOXA9*, and *HOXA10*) and are associated with high expression of the HOX cofactor *MEIS1*. At the molecular level, at least two distinct mechanisms have been involved in KMT2A-X leukemogenic properties and the deregulated expression of KMT2A-X target genes (Figure 1). On the one hand, the first 145 N-terminal amino acids of KMT2A interact with MEN1 and LEDGF to bind KMT2A target genes.[5] On the other hand, most fusion partners of KMT2A belong to the transcription elongation machinery leading to the active recruitment of various factors including (i) the P-TEFb complex (comprising CDK9), which phosphorylates RNA polymerase II; and (ii) the histone methyltransferases DOT1L and NSD1, which catalyze H3K79me3 and H3K36me2 marks deposited in the body of actively transcribed genes. This

creates an active gene transcription elongation environment at KMT2A-X target genes (e.g. *HOX* genes), which is reinforced by the recognition of acetylated lysines on histones at important oncogene loci (e.g. *MYC*) by the BET proteins including BRD4. Based on these dependencies, small molecule inhibitors of DOT1L, of BRD4 and of the interaction between KMT2A and MEN1 have been developed.[6-8]

Other alterations of KMT2A function are observed. In some instances reciprocal X-KMT2A fusions were shown to contribute to leukemogenesis in murine model (e.g. *AFF1-KMT2A* cooperation with *KMT2A-AFF1*[9]). *KMT2A* partial tandem duplications (*KMT2A*-PTD) are also found in AML and both murine modeling and human genetics indicate that *KMT2A*-PTD requires additional mutations to induce *bona fide* leukemia.[10,11]

Wildtype NUP98 is part of the nuclear pore complex, a large structure of ~30 proteins at the nuclear membrane which provides a bidirectional channel allowing small ions and peptides to diffuse and larger molecules (mRNA and proteins) to be actively transported by carriers. NUP98 is different from other nucleoporins as it contains multiple GLFG repeats allowing interaction with CREBBP/EP300 and it can be found throughout the nucleus. Nup98 was reported to be involved, together with Rae, in cell cycle progression and mitotic spindle regulation.[12] Notably, NUP98 is found at sites of actively transcribed genes presenting the H3K4me3 mark and is involved in cell cycle and development.[13] NUP98 is also involved with wildtype KMT2A and NSL in complexes regulating *HOX* gene expression.[14]

In leukemia, NUP98 is recurrently fused with over 30 different partners (including NSD1, KDM5A, but also homeodomain proteins such as HOXA9, HOXC11, HOXD11 or HOXD13). These fusions (termed NUP98-X here) result from chromosomal alterations that are frequently undetected by conventional cytogenetics due to the location of the *NUP98* gene close to the telomere of the short arm of chromosome 11 (11p15).[15,16] In the case of chimeras between NUP98 and homeodomain proteins, the GLFG repeats of NUP98 generally replace the transactivation domain. To date, all NUP98-X fusions have been

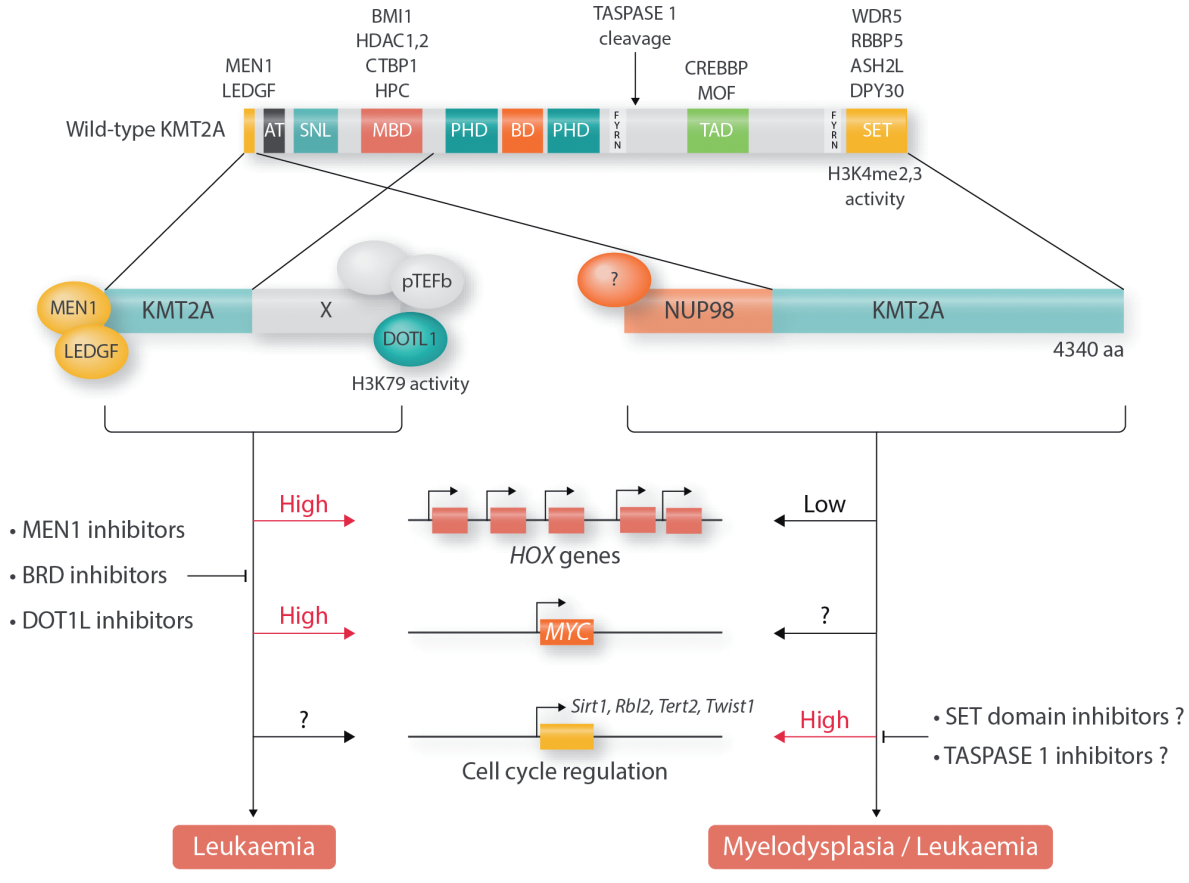

Figure 1. Molecular mechanisms associated with KMT2A-X and NUP98-KMT2A fusions. Schematic representation of the functional domains of wildtype KMT2A, KMT2A-X (where X corresponds to the fusion partners), and NUP98-KMT2A and the molecular mechanisms associated with leukemic transformation. While KMT2A-X fusions are associated with high expression (High) of *HOX* genes and *MYC*, NUP98-KMT2A is associated with low *HOX* genes (Low) and its regulation on *MYC* expression remains unknown (?). In mice, NUP98-KMT2A transformation is associated with high expression of cell cycle-associated genes (e.g. *Sirt1*, *Rbl2* and *Tert2*). Therapeutic targeting developed for KMT2A-X leukemia includes MEN1, DOT1L and BRD4 inhibitors. Whether small-molecule inhibitors of SET domain or TASPASE 1 activities would be efficient in NUP98-KMT2A leukemia is unknown. AT: AT-hook domain; SNL: speckled nuclear localization domains; MBD: MENIN 1-binding domain; PHD: plant homeodomain finger domain; BD: bromodomain; FYRN and FYRC: phenylalanine/tyrosine-rich-N- and C-terminal domains; TAD: transactivation domain; SET: SET methyltransferase activity domain.

associated with high *HOX* gene expression. Similarly to wildtype NUP98, the NUP98-HOXA9 fusion also interacts with wildtype KMT2A through the second GLFG repeat of NUP98 and KMT2A is important for the recruitment of NUP98-HOXA9 to the *HOXA* locus and NUP98-HOXA9-dependent *HOXA* genes expression.[17]

Fisher *et al.* performed functional modeling of a peculiar *NUP98-KMT2A* alteration, resulting from an inv(11)(p15;q23) characterized in two AML patients and leading to the fusion of *NUP98* exon 13 to *KMT2A* exon 2. The predicted *NUP98-KMT2A* fusion encodes a 4,340 amino acid protein lacking the MEN1-interacting domain but containing most of KMT2A including the H3K4 methylation SET domain, as opposed to KMT2A-X fusions (Figure 1). As the reciprocal *KMT2A-NUP98* fusion transcript (between exon 1 of *KMT2A* and exon 14 of *NUP98*) was detected in only one of the two original patients,[2] the hypothesis was that the *NUP98-KMT2A* fusion may represent the important disease driver. Fisher *et al.* achieved this *tour de force* through the development of a novel inducible NUP98-KMT2A transgenic mouse model.

The authors demonstrate the expansion and increased competitiveness of NUP98-KMT2A-expressing hematopoietic progenitor cells ($Lineage^-Sca1^+Kit^+$ cells) and concomitant cell cycle abnormalities without significant changes in the relative distribution between long-term hematopoietic stem cells and multi-potent progenitors. Upon long-term NUP98-KMT2A expression, mice succumbed to lethal myelodysplasia or AML. The median latency for development of a hematopoietic malignancy in inducible NUP98-KMT2A mice was rather long (>1 year) and sublethal irradiation to generate DNA damage accelerated disease. Together with the observation of additional mutations in other human *NUP98*-rearranged[15] or *KMT2A*-PTD,[11] this strongly suggests that cooperating mechanisms are required for induction of full-blown AML by NUP98-KMT2A. Interestingly, however, the co-expression of Flt3-ITD did not accelerate the disease in this inducible NUP98-KMT2A model, suggesting different cooperating networks as compared to the NUP98-NSD1 fusion.[18]

Inducible models allow elegant and formal testing of whether continuous expression of the driver oncogene is required for leukemia maintenance by removing doxycycline treatment in diseased animals. Previously, full dependence of AML on *KMT2A-MLLT3* expression was observed using a similar model.[19] Here, however, ceasing the doxycycline treatment in recipients of inducible NUP98-KMT2A cells did not abrogate the disease progression in all the mice. The authors suggest that this may result from a "leak" of residual NUP98-KMT2A expression inherent to this inducible system. In this regard, given that KMT2A-X and NUP98-KMT2A fusion transcript expression is controlled by different endogenous regulatory elements - KMT2A and NUP98 promoters, respectively - significantly different levels of fusion expression could be required for leukemia induction and maintenance. It remains to be formally tested whether NUP98-KMT2A expression is essential for the maintenance of already transformed leukemic cells in all cases.

At the molecular level, inducible NUP98-KMT2A murine leukemia cells, similarly to NUP98-KMT2A patient's leukemic cells,[2] do not show significant upregulation of *HOX* genes as compared to control cells or leukemia from two retroviral models of KMT2A fusions (KMT2A-ENL and KMT2A-MLLT3). Gene expression analyses in transgenic mouse embryonic fibroblasts confirmed cell cycle deregulation and further demonstrated a block in induction of senescence. Notably, a subset of cell cycle- and senescence-associated genes deregulated by transgene induction in mouse embryonic fibroblasts was also found to be deregulated in murine hematopoietic progenitor cells (e.g. *Sirt1*, *Rbl2*, *Tert2*). These data suggest that NUP98-KMT2A does not transform hematopoietic progenitor cells through aberrant expression of *HOX* genes and cofactors but through an alternative mechanism associated with a defective cell cycle checkpoint. Notably, this is further supported by the absence of significant cell cycle perturbation in inducible NUP98-KMT2A cells mediated by small MEN1 or BRD inhibitors, as opposed to their effects on KMT2A-MLLT3 cells.

Three NUP98 fusion partners (NSD1, NSD3, and KMT2A) have a SET domain and another partner is a known interactor of SET-containing proteins (SETBP1) with histone methyltransferase function. Although additional genome-wide chromatin analyses will be required to assess H3K4me3 profiles and NUP98-KMT2A DNA binding sites in NUP98-KMT2A cells, it could be hypothesized that aberrant deposition of H3K4me3 at NUP98 target genes enhances or ectopically creates promoter activities. More generally, it also remains to be determined: (i) whether the NUP98 or KMT2A moiety controls the identity of the target genes; (ii) whether dimerization is required for transformation as for other KMT2A-X fusions;[20] and (iii) whether the location of NUP98-KMT2A at the nuclear pore, reported to be in close proximity to the loci of actively transcribed cell cycle regulators, in part controls the identity of the target genes in a cell context-dependent manner.

Together, the identification of transcriptional targets of NUP98-KMT2A represents a first step toward the development of novel therapeutic strategies. Based on the protein structure, the NUP98-KMT2A transforming properties could depend on cleavage by TASPASE 1 and SET domain catalytic activity. As interference with these activities has been proposed,[21] future assessment of the efficacy and specificity of targeted therapies could be of interest in these human leukemias.

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T-cell and NK-cell neoplasms of the gastrointestinal tract – recurrent themes, but clinical and biological distinctions exist

Elaine S. Jaffe

Hematopathology Section, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.

E-mail: ELAINE S. JAFFE - ejaffe@mail.nih.gov

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The history of intestinal T-cell lymphomas begins with the early work of Peter Isaacson and Dennis Wright who described cases of "malignant histiocytosis" of the intestine that they linked to malabsorption and ulcerative jejunitis.[1] Subsequent work showed that "malignant histiocytosis of the intestine" was a form of T-cell lymphoma, later named enteropathy-associated T-cell lymphoma (EATL).[2] Since then, we have come to understand the distinction between EATL, closely linked to celiac disease, and monomorphic epitheliotropic T-cell lymphoma (MEITL), formerly EATL type II (Figure 1).[3] The work of Isaacson and Wright shaped the modern classification of both T-cell and B-cell intestinal lymphomas, giving us not only EATL, but also mucosa-associated lymphoid tissue (MALT) lymphoma. Sadly Dennis Wright passed away on April 08, 2020 at the age of 88.

Most cases of intestinal T-cell lymphoma were highly aggressive, but in the 1990s there was a series of reports of low-grade intestinal T-cell neoplasms, some of which mimicked lymphomatous polyposis.[4-8] The nature of this rare form of T-cell lymphoma was better defined in subsequent reports,[9,10] and incorporated into the Revised 4th Edition of the World Health Organization (WHO) classification[3] as a provisional entity under the term indolent T-cell lymphoproliferative disorder of the gastrointestinal tract (ITLPD-GIT) (Figure 1). Most patients had a chronic, relapsing clinical course, although in both of the above series late instances of large-cell transformation were described.[10,11]

In the current issue of *Haematologica*, Soderquist *et al.* expand our knowledge regarding the immunophenotypic spectrum of ITLPD-GIT and provide new insights into its molecular pathogenesis.[12] As with prior reports, all cases were derived from αβ T cells with an equal proportion of cases expressing either CD4 or CD8. One case each had either a double-positive or double-negative phenotype. The authors also examined the expression of T-bet (TBX21) and GATA3, but any conclusions regarding the functional or clinical significance of these markers, which have been examined more extensively in nodal peripheral T-cell lymphomas,[13] remain premature.

This study confirms the importance of alterations in