A tale of two genes: a new connection between *NIPBL* and *NPM1* in acute myeloid leukemia

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Acute myeloid leukemia (AML), the most common acute leukemia in adults, is defined by few somatic mutations in myeloid stem cells which drive abnormal proliferation and inhibit normal differentiation.[1] The overall survival rate of adults with AML remains low despite current front-line induction therapy using cytarabine and an anthracycline followed by a hematopoietic stem cell transplant in first remission for high-risk patients.[2] Defining the molecular mechanisms by which AML-associated somatic mutations drive leukemogenesis will illuminate new key pathways for therapeutic intervention to improve patient outcomes.

One of the most frequently mutated genes in adult AML is nucleophosmin (*NPM1*) which encodes a chaperone protein crucial to genomic stability, protein synthesis, and cell cycle regulation. Wildtype NPM1 localizes primarily to the nucleolus, but has the ability to shuttle rapidly between the nucleus and the cytoplasm.[3] About one third of primary adult AML cases with a normal karyotype harbor an oncogenic *NPM1* mutation, often in the C-terminal nuclear export signal (NES) that causes aberrant cytoplasmic translocation (*NPMc+*) and subsequent disruption of normal cellular regulation.[4,5] In zebrafish, a powerful model system for hematopoiesis, *NPMc+* mRNA injection into embryos results in cytoplasmic expression and an increase in both *cmyb* positive definitive hematopoietic stem cells and *spi1* positive myeloid progenitor cells, consistent with the phenotype of *NPMc+* adult AML

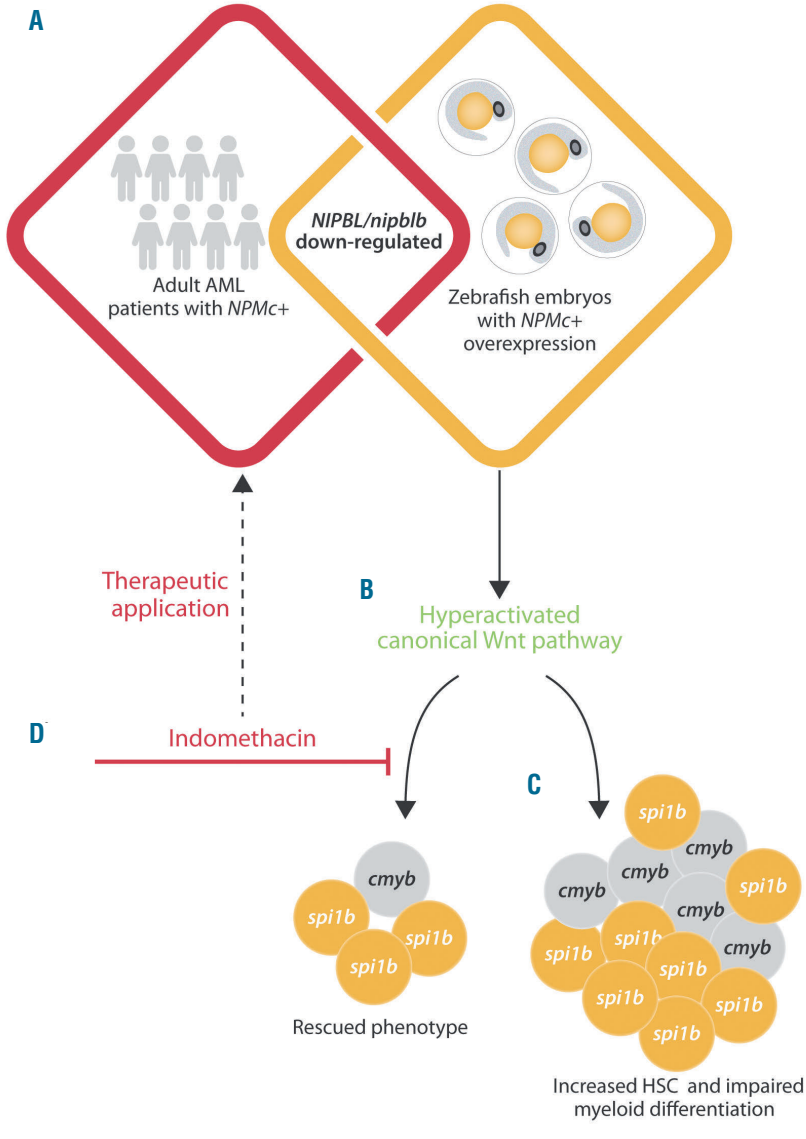

Figure 1. Model of leukemogenesis. (A) *NIPBL/nipblb* is downregulated in adult humans with acute myeloid leukemia (AML) and zebrafish embryos, respectively, harboring the *NPMc+* mutation. (B) *NPMc+* and *nipblb* downregulation drives hyper-activation of the canonical Wnt pathway. (C) Hyper-activation of the canonical Wnt pathway leads to an accumulation of *cmyb* hematopoietic stem cells (HSC) and *spi1b* myeloid progenitors. (D) The phenotype is rescued upon treatment with the Wnt pharmacological inhibitor indomethacin, a possible new approach to the treatment of *NPMc+* AML.

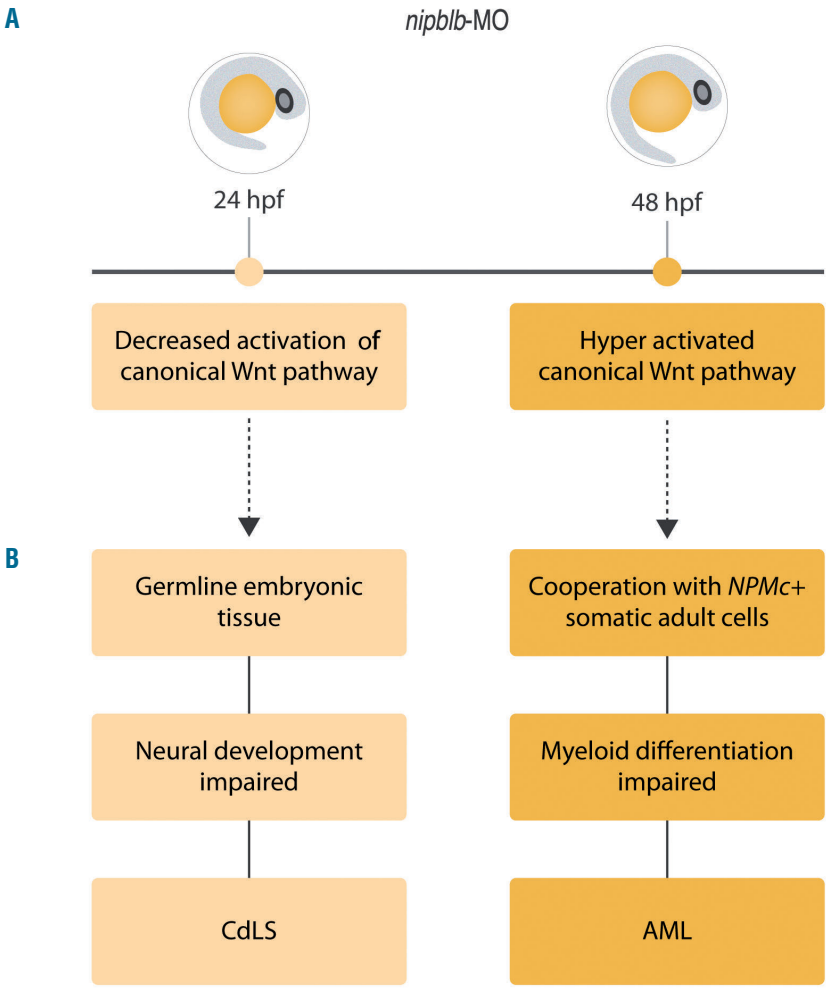

Figure 2. The dual role of NIPBL. (A) Zebrafish embryos were treated with *nipblb*-MO, an antisense oligonucleotide morpholino targeting the ATG region of nipblb. Twenty-four hours post-fertilization (hpf) there was a decrease in canonical Wnt pathway activation; however, at 48 hpf the pathway was hyperactivated, suggesting that *nipblb* plays a dual role in canonical Wnt pathway regulation. (B) When *NIPBL* is mutated in human germline embryonic tissue there is a decrease in canonical Wnt signaling, which leads to impaired neural development and progression to Cornelia de Lange syndrome (CdLS). When *NIPBL* is downregulated in cooperation with *NPMc+* mutation in somatic adult cells there is hyper-activation of the canonical Wnt signaling pathway, which leads to impaired myeloid differentiation and progression to acute myeloid leukemia (AML).

patients.[6] Subsequent injection of *dkk1b* mRNA, an inhibitor of the Wnt signaling pathway, rescues the increase of *spi1* myeloid progenitor cells suggesting *NPMc+* altered hematopoiesis is Wnt-dependent.[7] The canonical Wnt/β-catenin signaling pathway has been shown to play a role in the development of AML. When constitutively active β-catenin is expressed in normal primary human CD34$^+$ stem cells, the stem cells maintain high levels of CD34 expression and have impaired myelo-monocytic differentiation.[8] Analysis of a cohort of AML patients in the study by Simon *et al.* revealed several samples with aberrant expression of LEF-1, a transcription factor in Wnt signaling, and constitutive expression of the Wnt family members Wnt-1 and Wnt-2b.[8] These findings are consistent with a role for the Wnt signaling pathway in the development and progression of AML.

In this issue of *Haematologica*, Mazzola *et al.* report specific downregulation of *NIPBL/nipblb* in a cohort of adult patients with *NPMc+* AML and in *NPMc+* zebrafish embryos, respectively, and sought to understand the role of this cooperation in AML (Figure 1A).[9] Wildtype NIPBL is a cohesin-loading factor essential to cohesin complex function in the maintenance of the genome, specifically in replicating chromatids and DNA damage repair.[10] *NIPBL* is one of five cohesin complex genes mutated in association with Cornelia de Lange syndrome, a rare developmental disorder with varying genomic landscapes and phenotypic expression. *NIPBL* is the most commonly mutated gene in patients with Cornelia de Lange syndrome and, when its expression is low, confers the most severe phenotype.[11] When *nipblb* is knocked down in zebrafish there is a significant downregulation of *ccnd1*, a direct target of the Wnt signaling pathway.[12] *CCND1* is also downregulated in almost half of Cornelia de Lange syndrome patients with mutated *NIPBL*. This suggests that the most severely affected patients have low expression of *NIPBL* and subsequently low Wnt signaling that likely contributes to the disease phenotype.[12] This is in contrast to the increased Wnt signaling associated with *NPMc+* adult AML. Cohesin mutations have been identified in patients with AML although they are usually determined to be secondary events that contribute to clonal expansion rather than acting as the driving oncogenic mutation.[13] The data reported in this issue of *Haematologica* are intended to shed light on the cooperation between *NIPBL* and *NPMc+* in adult AML.[9]

To understand the role of *NIPBL* downregulation in *NPMc+* AML patients, Mazzola *et al.* used zebrafish to study the effects of these genetic alterations on hematopoietic stem cells and myeloid progenitors, cell populations that are increased in patients.[5,9] Embryos injected with a *nip-*

blb antisense oligonucleotide morpholino (*nipblb-MO*) showed an increase in *spi1b* myeloid progenitors but did not show an increase in hematopoietic stem cells. However, when embryos were injected with *NPMc+*, there was an increase in both *spi1b* myeloid progenitors and *cmyb* hematopoietic stem cells, which suggests *nipblb* downregulation cooperates with *NPMc+* as a secondary driver of impaired myeloid differentiation (Figure 1C).[13] To further elucidate the relationship between *NIPBL* downregulation and *NPMc+*, Mazzola *et al.* focused on the downstream pathway these genes have in common: canonical Wnt/β-catenin signaling. They found that this pathway was hyper-activated in *NPMc+* embryos and in embryos with *nipblb*-MO at 48 hours post fertilization (hpf) (Figure 1B). A dose-response assay demonstrated that subcritical doses of *nipblb*-MO or *NPMc+* given individually did not increase Wnt activation but co-injection of subcritical doses of *nipblb*-MO and *NPMc+* did, which supports the hypothesis that *nipblb* and *NPMc+* cooperate to drive hyper-activation of the canonical Wnt/β-catenin signaling pathway. Treatment with indomethacin, a pharmacological inhibitor of Wnt, rescued the increased hematopoietic stem cell and myeloid progenitor phenotype in *nipblb*-MO and *NPMc+* embryos, thus confirming that hyper-activation of the Wnt signaling pathway contributes to impaired myeloid cell differentiation, an observation also made in AML patients (Figure 1D).[8,9,14] The authors suggest that Wnt/β-catenin inhibitors, such as indomethacin, may be an applicable new therapeutic approach to treating *NPMc+* AML patients. It is important to note that canonical Wnt pathway activation in *NPMc+* AML patients in the cohort studied here was not significantly increased in patients with decreased *NIPBL* expression compared to the activation in those with normal or increased *NIPBL* expression. Larger cohorts and extensive Wnt pathway activation tests will be necessary before a clinical recommendation of indomethacin treatment can be made.

The study published by Mazzola *et al.* in this issue of *Haematologica* demonstrated that *nipblb* downregulation in zebrafish embryos induced Wnt pathway hyper-activation at 48 hpf. Interestingly, a previous study by the authors of this publication showed that at 24 hpf *nipblb* downregulation actually decreased Wnt pathway activation (Figure 2A).[12] Taken together, these studies propose two roles for NIPBL in the regulation of canonical Wnt signaling. In one situation, early downregulation of *NIPBL* in germline embryonic tissue initiates impaired neural development due to decreased Wnt pathway activation, which leads to the clinical presentation of Cornelia de Lange syndrome. Conversely, downregulation of *NIPBL* as a secondary event to *NPMc+* confers hyper-activation of the Wnt pathway in hematopoietic stem cells and early myeloid progenitors which impairs normal hematopoietic differentiation and induces progression to AML (Figure 2B).[9,15] The report in this issue of *Haematologica* tells a new tale of NIPBL and its cooperation with NPM1 in AML, thereby leading us to a greater understanding of the underlying molecular network that contributes to the disease.

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