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EDITORIAL

Nucleophosmin mutations in acute myeloid leukemia in children

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The paper by Mullighan *et al*.[1] in this issue of *Leukemia* joins a growing list of studies examining the frequency and clinical significance of mutations in the nucleophosmin (*NPM1*) gene in acute myeloid leukemia (AML) in children. The gist of their findings is that *NPM1* mutations are distinctly less common in pediatric leukemia than in adults, and are seen almost exclusively in cases with normal cytogenetics (NC). Leukemias with *NPM1* mutations tend to occur in older patients and to have monocytic features, and their leukemic blasts lack the expression of stem cell markers such as CD34 and CD133. The observations in this study confirm those of a number of recent publications which show that nucleophosmin mutations are rare in pediatric AML (5–6%) but, when they do occur, are associated with NC and older age.[2–4] In two studies from Asia, the incidence of *NPM1* mutation in AML appeared to be particularly low (0–2%),[5,6] suggesting racial variation in the susceptibility to this type of mutation.

The clinical and laboratory attributes of adult AML with nucleophosmin mutations have been well characterized and in many ways resemble the childhood cases. As in pediatric AML, *NPM1* mutations occur predominantly in AML with NC (AML-NC), increase with age and occur more often in females (see Falini *et al*.[7] for review). The laboratory similarities include an association with FLT3 internal tandem duplications (ITD) and a lack of expression of CD34. In adults, these mutations are associated with increased marrow blast counts, higher white blood cell and platelet counts, high serum lactate dehydrogenase levels, lymphadenopathy and gingival hypertrophy. Racial differences in the incidence of *NPM1* mutations in adult AML have not been reported.

Nucleophosmin is quite an intriguing molecule. It is a ubiquitously expressed protein with a remarkable array of imputed functions (see Grisendi *et al*.[8] for review). While it resides in the nucleolus, it shuttles rapidly between the cytoplasm and the nucleus, serving as a molecular chaperone for both nucleic acids and proteins. It is thought to play a role in extremely diverse cellular processes including ribosomal biogenesis, cell proliferation, genomic stability and apoptosis (via interactions with p53 and ARF), and histone and nucleosome assembly.

The involvement of nucleophosmin in malignant disease is equally complicated.[8] The *NPM1* mutations seen in AML-NC occur in exon 12 and result in the loss of two key tryptophans, thereby creating a new nuclear export signal and causing the protein to be trapped in the cytoplasm; indeed, immunohistochemical localization of NPM1 protein to the cytoplasm accurately predicts the presence of such mutations in AML.[9] These mutations have been reported to occur in up to 60% of adult cases of AML-NC, making them the most common mutation identified so far in this cytogenetic category. *NPM1* is also involved in a variety of chromosomal translocations that result in the fusion of the N-terminal portion of the NPM1 protein to different partners, creating chimeric proteins such as NPM1-RARα in variant forms of acute promyelocytic leukemia and NPM1-ALK in anaplastic T-cell lymphomas. Furthermore, NPM1 is overexpressed in many solid tumors, and yet fibroblasts deficient in NPM1 develop aneuploidy in culture and are more readily transformed by oncogenes.[10] So can we say that *NPM1* is a proto-oncogene or a tumor-suppressor gene, or is NPM1 a novel protein with features of both? Although we still have much to learn about the role(s) of NPM1 in malignancy, it is tempting to speculate that the abnormal protein seen in AML-NC is blocking a normal tumor-suppressor function of NPM1 by oligomerizing with wild-type protein, retaining it in the cytoplasm and blocking some key shuttling/chaperone activity.

An interesting feature of the Mullighan study was their analysis of the gene expression profiling of their pediatric cases with *NPM1* mutations. Although the number of cases analyzed was small, they observed a fairly homogeneous gene signature that distinguished them from other cases of AML-NC, and resembled previous expression profiles reported in adult cases with nucleophosmin mutations.[11] A notable feature of those profiles is a striking upregulation of several *HOX* genes, a phenomenon also seen in acute leukemias with mixed lineage leukemia (MLL) rearrangements.

This observation is important, as *HOX* genes play a key role in both normal and malignant hematopoiesis (see Eklund[12] for review). The *HOX* family of homeobox genes includes some 38 members, organized in four separate clusters (A, B, C and D) located on different chromosomes. A number of *HOXA* genes and, to a lesser extent, *HOXB* genes are normally expressed in primitive hematopoietic cells, where they appear to have important functions in cell proliferation and self-renewal. Furthermore, a number of *HOXA* and *HOXB* genes are frequently overexpressed in human AML, and have been suggested to have both diagnostic and prognostic value in this disease.[13] Although *HOX* genes have been reported to be activated in several subtypes of acute leukemia, they have been shown to be uniformly upregulated in cases bearing *MLL* fusion genes.[14] A number of *HOX* genes have been shown to be potent oncogenes, and readily cause AML when retrovirally overexpressed in murine bone marrow cells.[15]

In this study, the authors directly compared the expression profiles of cases with *NPM1* mutations with those of pediatric AML cases bearing *MLL* rearrangements, and make the intriguing observation that while several *HOXA* genes are upregulated in both types of leukemia, a number of *HOXB* genes are activated only in the cases with *NPM1* mutations. These findings raise some key issues. Firstly, they would appear to render moot the previous debate about the prognostic value of *HOX* gene expression, as it now seems clear that *HOX* genes are upregulated in AML cases with favorable outcomes (*NPM1* mutations) and with unfavorable outcomes (*MLL* fusions). Secondly, they raise the question as to whether *HOX* genes are mediating the leukemogenicity of *NPM1* mutations. In the case of *MLL* fusion leukemias, the evidence for this is mixed. In one study, one particular *MLL* fusion gene was unable to induce leukemia in cells lacking *HOXA9*, suggesting that this homeobox gene was crucial for *MLL*-fusion-gene-induced leukemias.[16] However, another study showed that a different *MLL* fusion could still cause leukemia in murine cells deficient in *HOXA9*, but that the phenotype of the leukemia was altered.[17] Whether the *HOX* genes that are upregulated in *NPM1*-mutant AML are key downstream players in these leukemias will be hard to prove, given the shear number of overexpressed *HOX* genes in

these cases. A third issue is the mechanism by which *HOX* gene expression is upregulated by the *NPM1* mutation. In the case of *MLL* fusion genes, the mechanism seems clearly to be due to direct binding of *MLL* proteins to the *HOX* gene cluster, maintaining that genomic region in an open and transcribable chromatin configuration.[18] The mechanism in *NPM1* mutations is currently a mystery, but might perhaps be due to the capacity of nucleophosmin to affect histone and nucleosome assembly.

Returning to clinical matters, these mutations may provide a convenient and novel molecular marker for monitoring of minimal residual disease in cases with NC and no known fusion genes.[19] More importantly though, *NPM1* mutations appear to be a major new predictor of good outcome in adult AML-NC. Although AML cases with *NPM1* mutations show a greater frequency of FLT3 ITD, which carry a poor prognosis, those cases with *NPM1* mutations that lack FLT3 ITD have a favorable prognosis with a high complete response rate and improved event-free and overall survival.[20–23] Indeed, such cases may not benefit from bone marrow transplant (BMT) in first remission.[24] Such cases may therefore be treated with chemotherapy alone, reserving BMT for those who relapse. These observations require further validation, but if true, would be a major step forward in risk stratification and treatment decision-making in adult AML. Whether *NPM1* mutations carry prognostic value in pediatric AML is unclear. In the Mullighan study, there was no discernible difference in outcome in the six cases bearing these mutations. In the recent study from the Pediatric Oncology Group, they observed an improvement in event-free survival that did not quite reach statistical significance ($P = 0.051$).[4] Thus, *NPM1* mutations may confer a favorable prognosis in pediatric AML, but the rarity of such cases may prevent statistical validation. All in all, the ability to identify *NPM1* mutations in AML will likely have great clinical utility, and testing for these abnormalities seems destined to become part of the standard laboratory evaluation in acute leukemia in the very near future, although the optimal methodology for their detection remains to be determined. Given the distinct clinical and laboratory features of such leukemias, it may be time to modify the WHO classification scheme for AML and create a new category of AML-NC with *NPM1* mutation.[25]

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