Nucleophosmin Mutations in *De novo* Acute Myeloid Leukemia: The Age-Dependent Incidences and the Stability during Disease Evolution

Wen-Chien Chou,[1,2] Jih-Luh Tang,[1] Liang-In Lin,[4] Ming Yao,[1] Woei Tsay,[1] Chien-Yuan Chen,[1] Shang-Ju Wu,[1] Chi-Fei Huang,[1] Rong-Jing Chiou,[1] Mei-Hsuan Tseng,[1] Dong-Tsamn Lin,[2,3] Kai-Hsin Lin,[3] Yao-Chang Chen,[1,2] and Hwei-Fang Tien[1]

Departments of [1]Internal Medicine, [2]Laboratory Medicine, and [3]Pediatrics, National Taiwan University Hospital; and [4]Graduate Institute of Medical Technology, National Taiwan University, Taipei, Taiwan

Abstract

***Nucleophosmin* (*NPM*) mutations have been found in a significant proportion of adults with *de novo* acute myeloid leukemia (AML), especially in those of a normal karyotype. These results provide a basis for studies of the pathogenesis in this specific subgroup of AML. In this study, *NPM* mutations were analyzed in 173 Chinese patients of *de novo* AML, including adults and children. We found that *NPM* mutations were present in 19.1% of the overall population and 40.3% of those with a normal karyotype. Adults had a significantly higher incidence of *NPM* mutations than children [32 of 126 (25.4%) versus 1 of 47 (2.1%), $P < 0.001$]. *NPM* mutations were closely associated with normal karyotype ($P < 0.001$) and internal tandem duplication of *FLT3* ($P = 0.002$), but negatively associated with *CEBPA* mutations ($P = 0.032$) and expression of CD34 ($P < 0.001$) and HLA-DR ($P = 0.003$). Serial analyses of *NPM* mutations showed the mutation disappeared at complete remission, but the same mutation reappeared at relapse, except for one who lost the mutation at the second relapse, when new cytogenetic abnormalities emerged. None acquired novel mutations during the follow-up period. In conclusion, *NPM* mutations occur in an age-dependent fashion. Moreover, the findings that *NPM* mutations are stable during disease evolution and closely associated with disease status make it a potential marker for monitoring minimal residual disease.** (Cancer Res 2006; 66(6): 3310-6)

Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of hematologic malignancy. Despite that recurrent chromosomal abnormalities are present in a significant proportion of AML patients, leukemia cells from ~40% to 50% of the patients have a normal karyotype and lack a reliable biological marker, thus making difficult the investigation of the etiologies and monitoring of residual disease in some of the *de novo* AML patients (1).

Recently, *nucleophosmin* (*NPM*) exon 12 mutations, which resulted in shift of the NPM protein from the nucleus to cytoplasm, were found in ~30% of *de novo* AML patients (2–8). The mutations were even more prominent in those with a normal karyotype and were frequently associated with *FLT3* mutation. NPM protein shuttles between the nuclei and cytoplasm and is involved in ribosomal biogenesis (9, 10), centrosome duplication (11), and regulation of the functions of tumor suppressor proteins, such as p53 and alternative reading frame (12, 13). Although it is frequently a partner of translocations in various hematologic malignancies, such as anaplastic large cell lymphoma (*NPM-ALK*; ref. 14), acute promyelocytic leukemia (*NPM-RARα*; ref. 15), myelodysplastic syndrome, and AML (*NPM-MLF-1*; ref. 16), the contribution of the mutated NPM protein to the leukemogenesis remains undetermined. The frequent mutation of *NPM* in *de novo* AML might provide a basis for investigation of the pathogenesis and monitoring of residual disease of AML with a normal karyotype.

Most reports of *NPM* mutations came from Europe and focused mainly on adults (2–7). In the current study, we investigated the mutation in 173 *de novo* AML patients, a population of mixed Chinese adults and children, and correlated the results with clinical features, cytogenetics, immunophenotyping, and other genetic alterations. We also did sequential analyses on some patient samples during the clinical course to investigate the stability and pathogenetic role of *NPM* mutation in AML.

Materials and Methods

Patients and sample collection. The *NPM* mutation was analyzed on the bone marrow cells from 156 consecutive patients with *de novo* AML diagnosed based on the French-American-British Cooperative Group criteria (17) at the National Taiwan University Hospital from 1995 to 2000. We subsequently recruited another 17 children with AML diagnosed outside this period to increase the number of pediatric patients. The bone marrow mononuclear cells were collected by heparinization followed by Ficoll-Hypaque gradient centrifugation. The isolated cells were frozen and stored at −80°C.

Mutation analysis. Analysis of *NPM* exon 12 mutation was done as described by Falini et al. (2). The final volume for PCR reaction was 35 μL containing 200 ng DNA, 200 nmol/L deoxynucleotide triphosphate, 2 mmol/L $MgSO_4$, 140 nmol/L of each primer, and 1 unit of AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). PCR was done by heating at 95°C for 10 minutes, followed by 35 cycles of 95°C for 45 seconds, 49°C for 1 minute, and 72°C for 1 minute, with a final step for 10 minutes at 72°C. PCR products were electrophoresed on 2% agarose gels, purified and sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit, which contained AmpliTaq DNA polymerase FS (Applied Biosystems), on an automated ABI-3100 Genetic Analyzer (Applied Biosystems). Abnormal sequencing results were confirmed by at least two repeated analyses. The DNA extracted from the peripheral blood mononuclear cells of 13 healthy persons was used as normal controls.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Hwei-Fang Tien, Department of Internal Medicine, National Taiwan University Hospital, 100 Taipei, Taiwan. Phone: 886-2-23123456, ext. 3955; Fax: 886-2-23959583; E-mail: hftd@ha.mc.ntu.edu.tw.

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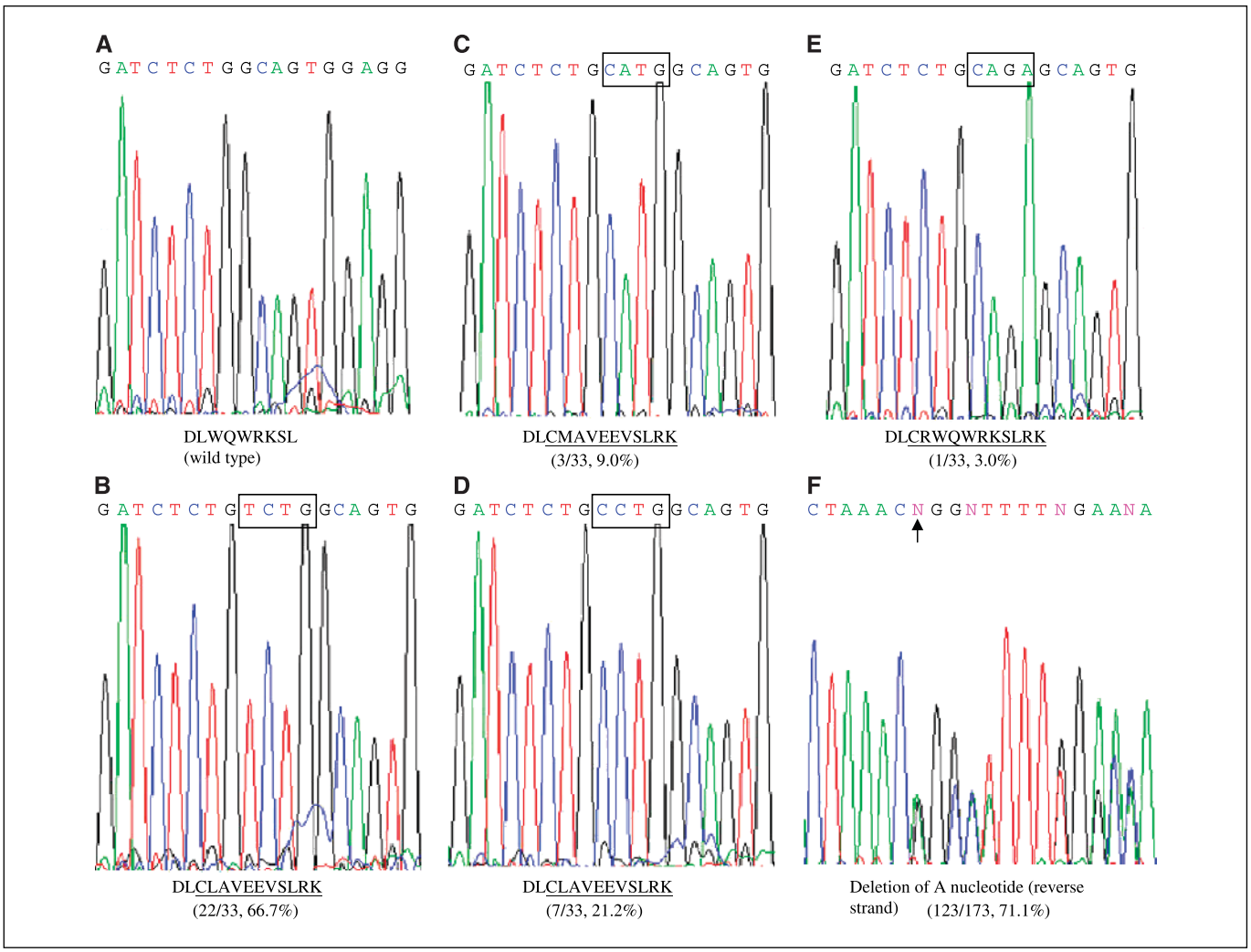

Figure 1. The *NPM* mutation patterns. The sequences of (*A-E*) were derived from single clones by cloning with pGEM-T easy kit. *A,* wild-type *NPM* sequence. *B* to *E,* mutation of *NPM. Boxed tetranucleotides,* insertion sequences between nucleotides 960 and 961 from the transcription start site. The underlying denotes the corresponding alteration of encoded amino acid sequences and incidences. *F,* polymorphism with deletion of T nucleotide at position 1,146. The sequences were read forward in (*A-E*), but backward in *F*.

Point mutations at codons 12, 13 (exon 1), and 61 (exon 2) of the *N-RAS* and *K-RAS* genes were analyzed by PCR on genomic DNA and direct sequencing (18). Methylation status of *SOCS1* (*suppressor of cytokine signaling-1*) and *SHP1* (*protein-tyrosine phosphatase containing 2 Src homology domains*), both encoding proteins functioning as negative regulators of signal transduction, was analyzed by methylation-specific PCR as described (19–24). Analyses of mutations of *CEBPA* and internal tandem duplication of *FLT3* (*FLT3*/ITD) were done according to previous studies (18, 25).

TA cloning. We chose the PCR products of all types of detected mutation and cloned with pGEM-T Easy TA cloning kit (Promega, Madison, WI), followed by sequencing to identify the specific mutation in a single allele.

Immunophenotyping. A panel of monoclonal antibodies to myeloid-associated antigens, including CD13, CD33, CD11b, CD15, CD14, and CD41a, as well as lymphoid-associated antigens, including CD2, CD5, CD7, CD19, CD10, and CD20, and lineage nonspecific antigens HLA-DR, CD34, and CD56 were used to characterize the phenotypes of the leukemia cells. Expression of surface antigens on the leukemia cells was shown by an indirect immunoalkaline phosphatase method (26) before 1998 and by flow cytometry thereafter.

Cytogenetic study. Bone marrow samples were aspirated into heparinized syringes and chromosomal analyses by G-banding method was done on bone marrow cells after 1 to 3 days of unstimulated culture as described previously (26).

Statistics. χ^2 Test was used to calculate the significance of association between *NPM* mutation and other discrete variables, such as expression of antigens, cytogenetics, mutation, or methylation of a specific gene, etc., and Mann-Whitney tests were used to compare continuous variables. Kaplan-Meier curve was calculated by SPSS software (Chicago, IL).

Results

***NPM* exon 12 mutations.** A total of 173 *de novo* AML patients, including 105 males and 68 females, with a median age of 41 years (range, 0-85 years), were analyzed. There were 126 adults and 47 pediatric patients (≤18 years old). Overall, the *NPM* mutation occurred in 33 (19.1%) of all *de novo* AML patients. There were four types of mutations, all involving the COOH-terminal portion of the transcript of *NPM* with a four-nucleotide insertion between positions 960 and 961. The frequencies and resulting changes of amino acid sequences are shown in Fig. 1*A* to *E*. All cases with the mutation were heterozygous and retained a wild-type allele. We also detected a polymorphism of nucleotide T deletion at position

1,146 in the 3′ untranslated region (Fig. 1*F*). The polymorphism was detected in 123 patients (71.1%), homozygous in 27 and heterozygous in 96, and in 11 of the 13 healthy persons.

Sequential studies of *NPM* mutations. *NPM* exon 12 mutations were serially studied in 41 patients, including 13 patients with and 28 patients without *NPM* mutation at diagnosis; 20 of the latter patients had 1,146(−T) polymorphism at the *NPM* gene (Table 1; Supplementary Table). None of these 41 patients acquired a new mutation after a median follow-up time of 16 months (range, 1-60 months), although eight of them showed karyotypic evolution and two had different clonal abnormalities at relapse. The *NPM* mutations disappeared at complete remission in all patients who harbored the mutations at diagnosis and had available DNA samples for analysis (Table 1). The same mutations as those at diagnosis were detected at first relapse in all five patients analyzed (patients 22, 55, 63, 129, and 147). However, no mutation was detected at second relapse in patient 63; at that time, new clonal karyotypic abnormalities were present. In one patient (patient 32), the mutation remained detectable after chemotherapy when bone marrow blasts were 7.2%. In the 20 patients with 1,146(−T) polymorphism but without mutation at the *NPM* gene, the nucleotide T deletion persisted at complete remission and also at relapse with the exception of one patient (patient 69) in whom the homozygous polymorphism turned to heterozygous status after allogeneic bone marrow transplantation (Supplementary Table). In all patients without *NPM* mutation at diagnosis and having subsequent samples for analysis, none acquired mutation at relapse.

Correlation of *NPM* mutations with clinical features and biological characteristics. One hundred and sixty-five patients

Table 1. Results of sequential studies of *NPM* mutations and chromosomal changes in the patients with the mutation at diagnosis

Case no.	Interval (mo)*	Status	Karyotype	*NPM* mutation†	Polymorphism‡
21		Diagnosis	N	+TCTG	−T
	1	CR	N	—	−T
22		Diagnosis	N	+TCTG	—
	4	Relapse	N	+TCTG	—
23		Diagnosis	N	+TCTG	—
	1	CR	ND	—	—
27		Diagnosis	N	+CTGC	−T
	56	CR	ND	—	−T
32		Diagnosis	N	+TCTG	−T
	2	PR	N	+TCTG	−T
38		Diagnosis	N	+CATG	—
	1	CR	ND	—	—
44		Diagnosis	N	+TCTG	−T
	4	CR	ND	—	−T
55		Diagnosis	+21	+TCTG	−T/−T
	3	CR	N	—	−T/−T
	18	Relapse	N	+TCTG	−T/−T
63		Diagnosis	N	+CTGC	—
	3	CR1	ND	—	—
	9	Relapse 1	ND	+CTGC	—
	19	CR2	ND	—	—
	19	CR2	ND	—	—
	8	Relapse 2	−7, t(12;18)	—	—
85		Diagnosis	N	+TCTG	—
	1	CR		—	—
120		Diagnosis	+4, +8	+TCTG	−T
	14	CR	N	—	−T
129		Diagnosis	N	+TCTG	−T/−T
	3	CR	N	—	−T/−T
	3	Relapse	N	+TCTG	−T/−T
147		Diagnosis	−Y	+CTGC	−T/−T
	25	Relapse	−Y	+CTGC	−T/−T

NOTE: Sequential analyses of *NPM* mutation were done on 41 patients. The data of the 20 patients without mutation but with polymorphism and the eight patients with neither mutation nor polymorphism were shown in the Supplementary Table. None of these 28 patients acquired *NPM* mutation at relapse although seven of them had karyotypic evolution at relapse and two had completely different chromosomal abnormalities from those at diagnosis (see the Supplementary Table).

Abbreviations: CR, complete remission; PR, partial remission; N, normal; ND, no data.

*Interval (months) between two consecutive studies.

†All patients with *NPM* mutations are heterozygous and have 4 bp insertions between nucleotides 960 and 961.

‡Deletion of T nucleotide at position 1,046 in the 3′ untranslated region.

had cytogenetic data. The *NPM* mutation was mainly seen in those of a normal karyotype (Table 2). Among the patients with a normal karyotype, 40.3% showed the *NPM* mutation, compared with 6.1% in those with chromosomal abnormalities. No *NPM* mutation was seen in patients with t(15;17), t(8;21), inv(16), or other recurrent chromosomal abnormalities, such as t(6;9), t(7;11), and deletions involving chromosome 5 or 7 (Table 2). The *NPM* mutation occurred much more frequently in adults than in pediatric patients [32 of 126 (25.4%) versus 1 of 47 (2.1%), $P < 0.001$]. It was infrequently detected in patients younger than 40 years [3 of 85 (3.5%); Fig. 2]. The lower incidence of the *NPM* mutation in children could not be explained by the lower rate of normal karyotype in pediatric patients than in adults [11 of 41 (26.8%) versus 56 of 124 (45.1%), $P = 0.038$] because the *NPM* mutation was still significantly less frequent in children than in adults when only the patients with a normal karyotype were considered [1 of 11 (9%) versus 26 of 56 (46.4%), $P = 0.021$]. Comparison of *NPM* mutations in different age groups revealed a gradient increase of mutation rate in the older patient groups (Fig. 2). Patients with AML M4 subtype had a higher *NPM* mutation rate than those with other subtypes, but the difference had not reached statistical significance yet (Table 2). *NPM* mutations were significantly associated with higher initial WBC counts, blast percentages, and platelet counts in the peripheral blood, but not sex, lactate dehydrogenase, and hemoglobin levels (Table 2).

We also analyzed mutations in *CEBPA, N-RAS*, and *K-RAS, FLT3*/ITD, and hypermethylation in *SHP1* and *SOCS1*, which were frequently detected in AML (21, 27). The comparison of these genetic or epigenetic alterations between the patients with and

Table 2. Clinical data, French-American-British Cooperative Group types, and cytogenetic changes in AML patients

Variant	Total (n = 173)	Mutation (n = 33, 19.1%)	Wild type (n = 140, 81.9%)	P
	n	n (%)	n (%)	
Age				<0.001
Adult	126	32 (25.4%)	94 (74.6%)	
Children	47	1 (2.1%)	46 (97.9%)	
Sex				0.230
Male	105	17 (16.2%)	88 (83.8%)	
Female	68	16 (23.5%)	52 (76.5%)	
Laboratory data				
LDH (units/L)		1,056	954	0.640
WBC/μL		39,700	21,240	0.031
Blast (%)		68	45	0.023
Platelet × 1,000/μL		57	34.5	0.024
Hemoglobin (g/dL)		8.4	8.0	0.146
FAB				0.094*
M_0	4	0 (0.0%)	4 (00.0%)	
M_1	43	10 (23.3%)	33 (76.7%)	
M_2	64	13 (20.3%)	51 (79.7%)	
M_3	17	0 (0.0%)	17 (100.0%)	
M_4	26	8 (30.8%)	18 (69.2%)	
M_5	12	1 (8.3%)	11 (91.7%)	
M_6	0	0 (NA)	0 (NA)	
M_7	3	0 (0.0%)	3 (100.0%)	
Undetermined		1 (3)		<0.001†
Cytogenetic				
Abnormal				
t(15;17)	17	0 (0.0%)	17 (100.0%)	
t(8;21)	16	0 (0.0%)	16 (100.0%)	
Inv(16)	4	0 (0.0%)	4 (100.0%)	
t(7;11)	4	0 (0.0%)	4 (100.0%)	
t(6;9)	5	0 (0.0%)	5 (100.0%)	
del(7)	7	0 (0.0%)	7 (100.0%)	
del(5)	84	0 (0.0%)	4 (100.0%)	
Trisomy8	8	1 (12.5%)	7 (87.5%)	
Others	35	5 (14.3%)	30 (85.7%)	
Subtotal	98‡	6 (6.1%)	92 (93.9%)	
Normal	67	27 (40.3%)	40 (59.7%)	

Abbreviations: FAB, French-American-British Cooperative Group; LDH, lactate dehydrogenase; NA, not applicable.
*Comparing M_4 subtype and non-M_4 subtypes.
†Comparing normal karyotype and others.
‡Including one patient with del(7) plus del(5), and one with del(5) plus trisomy 8.

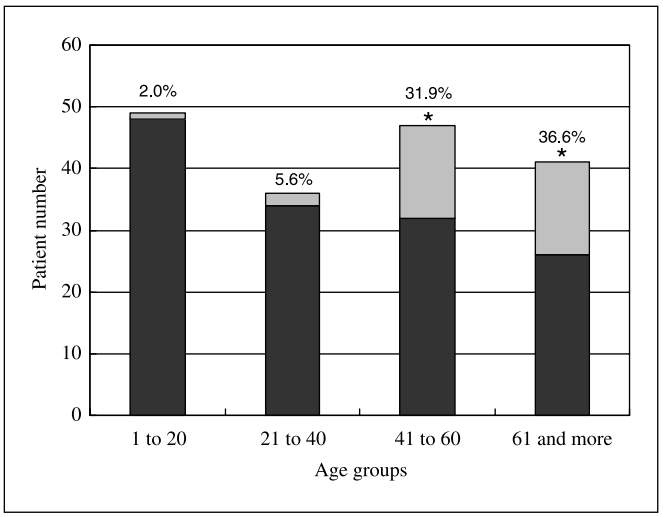

Figure 2. Age-dependent incidences of *NPM* mutation. *, $P < 0.001$, compared with age groups 0 to 20 and 21 to 40. Percentages above the columns represent the incidences of the *NPM* mutation in that specific group of patients ▭, mutation; ■, wild type.

without *NPM* mutation is summarized in Table 3. *FLT3*/ITD and *CEBPA* mutations were positively and negatively associated with *NPM* mutations, respectively. None of other genetic changes was associated with *NPM* mutation.

For immunophenotyping, the *NPM* mutation was associated with lack of expression of CD34 and HLA-DR on leukemia cells ($P < 0.001$ and $P = 0.003$, respectively; Table 4). Other markers, such as CD13, CD33, CD11b, CD14, CD15, CD19, CD10, CD7, CD56, and CD2, were not found to be significantly associated with *NPM* mutation status. We did not detect significant effects of the *NPM* mutation on the disease-free or overall survival whether *FLT3* mutation status was considered or not (data not shown), probably because of the small sample size.

Discussion

In this comprehensive analysis of *NPM* mutations in *de novo* AML patients, we found a remarkable difference in the incidence of *NPM* mutation between adult and pediatric patients (25.4% versus 2.1%). A recent study has reported a low incidence of *NPM* mutation in pediatric patients (7%; ref. 28). Verhaak et al. (6) showed that *NPM* mutations were significantly less frequent in adult patients younger than 35 years. Our current report is the first to present a side-by-side comparison of the *NPM* mutation rate in Chinese children and adults with AML. The difference in the incidence of *NPM* mutation between these two age groups could not be explained by the lower incidence of normal karyotype in the pediatric patients than in adults because the same finding could be shown although only the patients with a normal karyotype were considered (a mutation rate of 46.4% in adults versus 9% in children, $P = 0.021$). This is another proof that childhood leukemia can be very different from that of adults in pathogenesis and biological characteristics (1, 29).

The facts that *NPM* mutation is not present in leukemia with recurrent cytogenetic abnormalities and that the mutation disappears at complete remission imply a critical role of *NPM* mutation in leukemogenesis. Furthermore, we showed that none of the patients without the *NPM* mutation at diagnosis acquired the mutation during the follow-up period, suggesting that the *NPM* mutation might play little role in the progression of AML. In the sequential studies on a smaller population of patients, Boissel et al. (4) and Suzuki et al. (8) also found that none of their patients without *NPM* mutation at diagnosis acquired *NPM* mutation at relapse. Although the same *NPM* mutation as that detected at diagnosis could be identified again at first relapse in the *NPM*-mutated patients in our study, one patient lost the mutation at second relapse (patient 63; Table 1). The bone marrow blasts in this patient were 5.5% when the second relapse was diagnosed, but the relapse status was confirmed by the clonal chromosomal abnormalities of −7 and t(12;18), which were not seen at initial diagnosis. The disappearance of *NPM* mutation in this patient could be due to suppression of PCR amplification of mutant gene when there were only a small number of mutated cells within a major population of normal cells. Analysis by more sensitive quantitative real-time PCR may solve this problem. Another possible explanation was the emergence of a different clone of leukemia cells at that time. The presence of new chromosomal abnormalities −7, which was commonly seen in secondary leukemia, and the long interval (58 months) between initial diagnosis and this relapse (Table 1) supported the latter possibility. In the report of Boissel et al. (4), all 10 patients with *NPM* mutations at diagnosis still displayed *NPM* mutations at relapse. Of the 17 patients with *NPM* mutations at diagnosis reported by Suzuki et al. (8), 15 carried the same mutation at relapse. Two patients in that study lost the mutation at relapse; one of them acquired a chromosomal abnormality that was not present at

Table 3. Comparison of other genetic alterations between AML patients with and without *NPM* mutation

Variant	Total patients		*NPM*-mutated patients		*NPM*-wild patients		*P*
	No. studied	No. with alteration (%)	No. studied	No. with alteration (%)	No. studied	No. with alteration (%)	
CEBPA	168	26 (15.5)	32	1 (3.1)	136	25 (18.4)	0.032
FLT3/ITD	169	34 (20.1)	33	13 (39.3)	136	21 (15.4)	0.002
N-RAS	168	27 (16.1)	32	7 (21.8)	136	20 (14.7)	0.320
K-RAS	170	6 (3.5)	32	0 (0)	138	6 (4.3)	0.230
SHP1	128	89 (69.5)	30	25 (83.3)	98	64 (65.3)	0.061
SOCS1	80	45 (56.2)	15	6 (40)	65	39 (60)	0.371

NOTE: Genetic alterations are mutation in *CEBPA, N-RAS, K-RAS,* and *FLT3*/ITD, and hypermethylation in *SHP1* and *SOCS1*.

Table 4. Comparison of immunophenotyping between AML patients with and without *NPM* mutation

Variant	Percentage of patients with the antigen expression			*P*
	Total patients	*NPM*-mutated patients	*NPM*-wild patients	
HLA-DR	70.8	48.3	76.0	0.003
CD34	61.6	28.6	68.0	<0.001
CD13	86.6	89.7	85.9	0.595
CD33	92.2	100	90.5	0.089
CD11b	31.2	38.5	29.0	0.365
CD14	15.4	22.2	13.9	0.281
CD15	67.3	64.5	68.1	0.707
CD19	9.8	3.4	11.3	0.201
CD10	1.4	3.6	0.9	0.268
CD7	25.2	25.0	25.2	0.975
CD2	10.5	3.6	12.0	0.188
CD56	28.9	16.7	31.6	0.206

diagnosis and the other showed normal karyotype at both diagnosis and relapse. Together, these findings suggest that *NPM* gene status is stable in contrast to *FLT3*/ITD (8, 18) and that *NPM* mutation can be a good marker for monitoring minimal residual disease in AML.

We found that *NPM* mutations were mutually exclusive with *CEBPA* mutations but were highly associated with *FLT3*/ITD. Recent studies argue for a multistep pathogenesis of AML (30). The genetic events underlying AML pathogenesis appear to fall into two broadly defined complementation groups: One comprises mutations that confer proliferation and/or survival advantage to hematopoietic cells, such as *FLT3* and *RAS* mutations, and the other comprises mutations that affect transcription factors resulting in impaired hematopoietic differentiation, such as t(8;21)/*AML1-ETO*, inv(16)/*CBFβ-SMMHC*, and t(15;17)/*PML-RARα* fusions as well as *CEBPA* and *AML1* mutations (30, 31). Like *CEBPA, NPM* mutation is not present in AML with t(8;21) t(15;17) or inv(16) (25, 31, 32). The mutation of *NPM* results in cytoplasmic translocation of NPM protein, which is normally located in the nucleoli (33) where it may serve as a chaperone to prevent protein aggregation (34). Although the pathogenetic role of *NPM* mutation in AML is not clear, the findings that *NPM* mutation is inversely associated with *CEBPA* mutation and chromosomal abnormalities, t(8;21), t(15;17) and inv(16), suggest that mutant NPM protein may serve to impair differentiation of hematopoietic cells in the multistep pathogenesis model of AML, like *CEBPA* and *AML1* (6, 30, 31). Consistent with this hypothesis, a recent study on animal models revealed an indispensable role of NPM in embryonic hematopoiesis (35). In addition, haploinsufficiency of NPM in mice resulted in a syndrome mimicking human myelodysplastic syndrome, a hematologic disease of impaired differentiation (35). It was surmised that deletion of chromosome 5q commonly seen in myelodysplastic syndrome might be related to haploinsufficiency of NPM. Although there were no mutations at the exon 12 of *NPM* in >100 myelodysplastic syndrome patients tested (2), we cannot exclude the possibilities of haploinsufficiency by microdeletion of chromosome 5q involving this gene or a functional compromise by mutations of other genes in the same pathogenetic pathway.

In summary, *NPM* mutations were detected in 19.1% of a total of 173 *de novo* Chinese AML patients and 40.3% of those with a normal karyotype. Children had a significantly lower incidence of *NPM* mutations than adults. The mutation occurred infrequently in AML patients younger than 40 years. The same was also true if only the patients with normal karyotype were analyzed. The genetic changes underlying the childhood AML seem quite different from those in adult AML. Serial studies showed that the *NPM* mutation detected at diagnosis disappeared at complete remission and the same mutation reappeared at relapse. One patient lost the mutation at the second relapse. Whether this is due to an emergence of a new clone of leukemia cells or low sensitivity of the method to detect the mutation remains to be determined. No one acquired a new mutation during a median follow-up time of 16 months (range, 1-60 months), suggesting that the *NPM* mutation is probably an early event in the development of AML but may play little role in the progression of the disease. The findings that the *NPM* mutation was inversely associated with the *CEBPA* mutation and chromosomal abnormalities t(8;21), t(15;17), and inv(16), and was closely correlated with *FLT3*/ITD infer that the NPM mutants might impair the differentiation of hematopoietic cells. Further comprehensive studies on the biological effects of NPM mutants are needed to disclose the role of *NPM* mutations in the pathogenesis of AML and their interactions with other genetic alterations.

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