Nucleophosmin mutation in Southeast Asian acute myeloid leukemia: eight novel variants, *FLT3* coexistence and prognostic impact of *NPM1/FLT3* mutations

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ABSTRACT

NPM1 mutations were investigated in 400 Southeast Asian leukemia patients and were detectable in 105 cases (26.25%) of acute myeloid leukemia but in no cases of acute lymphoid leukemia or chronic myeloid leukemia. Eight novel and 5 known mutations were identified. All predicted novel proteins shared the last five amino acids *VSLRK* with the similar gain of nuclear exporting signal motif as known variants. Older age, high white blood cell and platelet counts, normal cytogenetics, and CD34-negativity were associated with *NPM1* mutation. *FLT3* mutation was more frequent in mutant *NPM1* than wild-type cases (56.8% *vs.* 25.6%) whereas RAS and *AML1* mutations were rarely found. Overall survival analysis based on the *NPM1/FLT3* mutational status revealed a better outcome for the *NPM1*-positive/FLT3–negative subgroup. We conclude that: i) *NPM1* mutation represents a common genetic hallmark in Southeast Asian acute myeloid leukemia with a normal karyotype; ii) *NPM1* mutants coexisted mainly with *FLT3* mutants, but not *RAS* or *AML1*; iii) FLT3 mutation had a negative prognostic impact on patients with mutant *NPM1*.

Key words: acute myeloid leukemia, *NPM1* mutation, *FLT3*, normal karyotype, Southeast Asia, novel mutations.

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease with distinct biological and prognostic characteristics.[1] Various clinical subsets associated with unique chromosomal translocations have been identified in AML and appear to be associated with specific prognostic outcome depending on the underlying chromosome abnormalities. Three cytogenetic risk groups, i.e. favorable, intermediate and unfavorable, are well recognized. The intermediate risk group is a heterogeneous group of patients lacking any specific karyotypic abnormalities the majority of whom have a normal karyotype. Nucleophosmin is a member of the nucleophosmin/nucleoplasmin (NPM) family of nuclear chaperones whose members have been found throughout the animal kingdom.[2] The NPM family has a diverse function in cellular processes such as genome stability, ribosome biogenesis, DNA duplication and transcriptional regulation.[3] *NPM1* is the most studied member of the NPM family as it plays an important role as a versatile partner in many chromosomal translocations.[4] Frameshift mutation at exon 12 of the *NPM1* gene is an alternative leukemogenetic mechanism rather than chromosomal translocations that was recently discovered in 2005.[5-7] *NPM1* mutation is now recognized as one of the most frequent mutations in AML patients with a normal karyotype in European countries.[8-10] The aim of this study was to evaluate the prevalence and type of *NPM1* gene mutations in Southeast Asian adult AML patients and to investigate the associated biological and clinical characteristics.

Design and Methods

Leukemia samples

Consecutive leukemia samples were obtained from 400 *de novo* AML, 30 acute lymphoid leukemia (ALL), and 30 chronic myeloid leukemia (CML) patients between 2000 and 2005. This study was part of a large leukemia project previously approved by the Ethical Committee for Human Research, Faculty of Medicine, Siriraj Hospital, Mahidol University. Morphological, immunophenotypic and chromosome analyses were performed according to the standard methods and karyotypes described according to the International System for Cytogenetic Nomenclature.[11,12] *FLT3* internal tandem duplication (ITD), *RAS* and *AML1* mutations were analyzed according to our previously described methods.[13-15]

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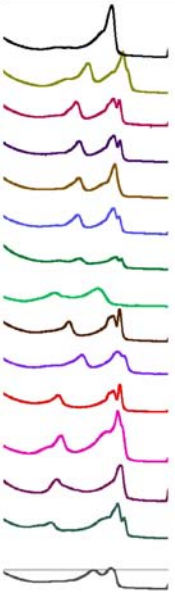
Table 1. Chromatograms of *NPM1* wild type and *NPM1* mutants.

Chromatogram	Type of mutation	Sequence				Protein	N. of cases %
	Wild type	GATCTC	TG	GCAG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLWQWRKSLstop	295/73.8
	Type A	GATCTC	TG **TCTG**	GCAG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLCLAVEEVSLRKstop	81/20.3
	Type B	GATCTC	TG **CATG**	GCAG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLCMAVEEVSLRKstop	5/1.3
	Type D	GATCTC	TG **CCTG**	GCAG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLCLAVEEVSLRKstop	7/1.8
	Type J	GATCTC	TG **TATG**	GCAG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLCMAVEEVSLRKstop	2/0.5
	Type DD-4	GATCTC	TG **TGTG**	GCAG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLCVAVEEVSLRKstop	1/0.3
	Type TH1	GATCTC	TG **TTCG**	GCAG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLCSAVEEVSLRKstop	1/0.3
	Type TH2	GATCTC	TG **TAAA**	GCAG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLCKAVEEVSLRKstop	1/0.3
	Type TH3	GATCTC	TG	GCAG **CGGC**	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLWQRLEEVSLRKstop	1/0.3
	Type TH4	GATCTC **CGCA**	TG	GCAG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLRMAVEEVSLRKstop	1/0.3
	Type TH5	GATCTC	TG **CACGCG**	AG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLCTRVEEVSLRKstop	1/0.3
	Type TH6	GATCTC	TG **CCAGAG**	AG	TGGAGG	AAGTCTCTTTAAGAAAATAG	DLCQRVEEVSLRKstop	1/0.3
	Type TH7	GATCTC	TG	GCAG	**TCCCTTTCTA**	AAGTCTCTTTAAGAAAATAG	DLWQSLSKVSLRKstop	1/03
	Type TH8	GATCTC **GTTTTGGCGA**	TG	G	AGG	AAGTCTCTTTAAGAAAATAG	DLVLAMEEVSLRKstop	1/0.3
	SNP							

Type A, B, D, J, and DD-4 were named as previously reported.[11,25] TH1-TH8 were novel mutations originated in this study. Red letters indicate nucleotide base insertion. Blue letters indicate tryptophans or the changing proteins at residues 288 and 290 in the wild-type and mutated NPM1 protein. Green letters indicate the NES motif L-xxx-(V, L, M)-xx-V-x-L. Underlined letters indicate the last five amino acids VSLRK.

Identification of NPM1 mutation

Mononuclear cells were isolated from the leukemia samples by Ficoll-Hypaque density-gradient centrifugation and genomic DNA extracted by the standard phenol-chloroform method. The polymerase chain reaction (PCR) amplification of the NPM1 gene exon 12 fragment was performed with the following oligonucleotide primers: forward (NPM11-F) (5'-ACCACATTTCTTTTTTTTTTTTCCAGGCT-3') and reverse (NPM12-R) (5'-CCTGGACAACATTTATCAAACACGGTA-3'). The total reaction volume of 25 μL contained 50 ng of genomic DNA, 2.5 mmol/L MgCl2, 0.4 mM dNTPs, 0.4 μM of each primers, 1 U of *Taq* DNA polymerase, and supplied buffer (Platinum® *Taq* DNA Polymerase, Invitrogen, Brazil).

PCR amplification was performed under the following steps: initial denaturation at 95 °C for 2 mins., 35 cycles at 94 °C for one min., 61°C for 30 secs. and 72°C for 30 secs. A final elongation proceeded at 72°C for 2 mins. Denaturing high performance liquid chromatography (DHPLC) analysis was carried out using the WAVE® Nucleic Acid Fragment Analysis System 3500HT with DNASep® HT cartridge technology (Transgenomic Inc, Omaha, NE, USA).

The optimized condition and temperature was predicted by the Navigator™ software. Approximately 5-10 μL of the crude PCR products were injected onto the DHPLC system and eluted under an optimized temperature at 55.5°C. DNA fragments were analyzed for changes in the chromatographic peak pattern. PCR products showing a change in the DHPLC pattern were identified as containing potential mutations and reamplified for sequencing analysis. The sequences were compared to the wildtype *NPM1* cDNA (GenBank Accession number, NM_002520).

Results and Discussion

Characterization of NPM1 mutations by DHPLC and sequencing

Wildtype chromatograms performed in one single peak were detected in 295 cases (73.75%) by DHPLC. Fourteen patterns of chromatograms that differed from the wildtype are shown in Table 1, thirteen of which were confirmed as *NPM1* mutations by sequencing while one pattern was found to represent a single nucleotide polymorphism (SNP). The SNP was detected in 9 patients (2.3%). None of the patterns of 30 ALL and 30 CML cases was found to be aberrant.

All mutated cases were heterozygous and retained a wild type allele. Similar to other studies from the western countries,[6-10] the most frequent mutation variant in the Southeast Asian population (81 cases, 20.3%) was a type A mutation which was a 4-bp TCTG insertion between the position 956 and 959, followed by type D (CCTG) in 7 cases (1.8%), type B (CATG) in 5 cases (1.3%), type J (TATG) in 2 cases (0.5%), and type DD-4 (TGTG) in one case (0.3%). Eight variants, Thai (TH)1 to TH8, were identified as novel mutations and each

novel mutation was detected in a separate patient, including a 4-bp insertion between the position 960 and 961 (type TH1, and TH2), between the position 964 and 965 (type TH3), and between the position 958 and 959 (type TH4). Mutation type TH5 and TH6 were a 6-bp insertion associated with a 2-bp deletion (ins6del2) at the position 960. Mutation type TH7 was a 9-bp insertion associated with a 5-bp deletion (ins9del5) at the position 965 and mutation type TH8 was a 10-bp insertion associated with a 6-bp deletion (ins10del6) at the position 958. Noticeably, all mutations resulted in a net of 4-bp addition either from insertion alone or associated with additional deletion. It is interesting that all eight novel *NPM1* variants from this study appeared to maintain the last five amino acid residues, *VSLRK*, and gain the nuclear exporting signal (NES) motif in much the same way as other previously reported variants.[6-9] Our findings indicated some common features of *NPM1* mutations among Southeast Asian patients and worldwide with respect to nucleotide base changes and their mutant proteins. The disruption of NPM1 nucleolar localization signal caused accumulation of NPM1 protein in the cytoplasm, possibly a critical step in malignant transformation.[16]

Associated genetic abnormalities and clinical parameters of patients with NPM1 mutations

Chromosomal aberrations were rarely seen in patients with *NPM1* mutations in this study (4.2%) and other studies.[7-10] Only 4 *NPM1* mutated cases had abnormal cytogenetics, i.e. del(9), inv(9), del(10), and inv(10). Chromosomal abnormalities are probably secondary events in the molecular pathogenesis of AML as cells with an abnormal karyotype could represent a subclone within the leukemic population with a normal karyotype. It is intriguing, however, that among the 4 AML cases with abnormal cytogenetics in our series, the abnormal chromosomes mainly involved chromosomes 9 and 10. The association of del(9q) with *NPM1* mutations has recently been recognized by Corbacioglu *et al.*[17] who reported the incidence of the *NPM1* mutation of 29% in 35 AML patients with del(9q). Although del(9q) was found in only 9 out of 400 AML cases in this study, we were able to identify one patient with del(9q) who also had *NPM1* mutation (11%). Because of the mutually exclusive nature of *NPM1* mutations and recurrent chromosomal abnormalities, Falini *et al.* recently proposed that AML patients with *NPM1* mutations or cytoplasmic NPM should be categorized as a distinct entity in the upcoming World Health Organization classification.[10]

With respect to additional genetic aberrations, three different types of genes were studied including *FLT3*, *AML1* and *RAS* (Table 2). *FLT3*-ITD mutation was detected in 56.8% of mutated *NPM1* cases compared to 25.6% of wild-type cases (p value <0.0001). Only one case in the mutated *NPM1* group and 25 cases in the wild-type *NPM1* group had RAS mutation. No AML1 mutation was found among the mutated *NPM1* group whereas 11 cases of *AML1* mutation were found in the wild-type *NPM1* group. The higher frequency of FLT3 mutations in cases with mutated *NPM1* suggests a possible pathogenic link between these two gene mutations. Mutated *FLT3* could induce chronic myeloid disorders in the murine model, but it alone is not sufficient to induce AML.[1] Additional mutation may be involved or needed in the pathogenesis of AML with FLT3 mutation. Although the role of *NPM1* mutation in AML is still not clear as there is currently no established animal model of AML with mutated *NPM1*, the coexistence of *NPM1* and *FLT3* mutations could suggest that the mutant NPM protein may serve to impair differentiation of hematopoietic cells in the same way as *AML1* and *CEBPA*. It is, therefore, possible that *NPM1* could co-operate with *FLT3*, but not *RAS* or *AML1*, in the multi-step pathogenetic model of AML.[1]

NPM1 mutations were particularly associated with specific clinical factors; for instance, a higher platelet count was one of the parameters. This was similarly observed by Thiede *et al.*[9] who suggested that blasts with *NPM1* mutation might retain a certain capacity for thrombocytic differentiation as demonstrated by *in vitro* experiments. Immunophenotypically, CD34-negativity and multi-lineage involvement, a common intrinsic feature of *NPM1* mutation that has already been reported, were also observed in this study.[7-10] Age-dependency was another *NPM1* mutation characteristic due to the significantly higher incidence found in adults than in pedi-

Table 2. Clinical parameters of acute myeloid leukemia patients carrying *NPM1* mutant (MT) and *NPM1* wild-type (WT).

Parameter	*NPM1-MT*	*NPM1-WT*	*p value*
Sex (F/M)	63/42	160/135	0.307[a]
Median age (years)	51	40	< 0.001[b]
Median WBC ($\times 10^9$/L)	47.0	25.4	< 0.001[b]
Median Hb (g/dL)	7.9	7.4	0.087[b]
Median Plt ($\times 10^9$/L)	57	39	0.002[b]
CD34$^+$ cells (%)	18.1	63.1	< 0.001[a]
FLT3	n=81	n=238	
FLT3-positive	46 (56.8%)	61 (25.6%)	
FLT3-negative	35 (43.2%)	177 (74.4%)	
RAS	n=54	n=153	
RAS-positive	1 (1.9%)	25 (16.3%)	
RAS-negative	53 (98.1%)	128 (83.7%)	
AML1	n=71	n =228	
AML1-positive	0	11 (4.8%)	
AML1-negaive	71 (100%)	217 (95.2%)	
Karyotype	n=95	n=273	
Normal	91 (95.8%)	146 (53.5%)	
Abnormal	4 (4.2%)	127 (46.5%)	
Age group	n=105	n = 295	
≤20	5 (9.4%)	48 (90.6%)	
21-40	23 (19.2%)	120 (80.8%)	
41-60	46 (35.7%)	129 (64.3%)	
≥60	30 (37.0%)	81 (63%)	
Median overall survival (months)			0.036
FLT3-positive	2.8	6.4	
FLT3-negative	14.9	10	

[a]χ^2 *test,* [b]*Mann-Whitney test.*

Table 3. Incidence of *NPM1* mutations in acute myeloid leukemia patients from various countries.

Country	N. of cases	N. of NPM1 mutation(%)[a]	Cases with cytogenetic data	N. of AMLcases with a normal karyotype	N. of NPM1 mutation among AML cases with a normal karyotpe (%)[b]
ASIA					
Taiwan					
Chou W *et al.* (2006)[18]	173 (adult & pediatric)	33 (19.1)	165	67	27 (40.3)
Japan					
Suzuki T *et al.* (2005)[21]	257 (adult)	64 (24.9)	209	97	46 (47.4)
China					
Zhang Y *et al.* (2007)[19]	28 (adult)	4 (14.3)	28	28	4 (14.3)
Yan L *et al.* (2007)[20]	156 (adult)	44 (28.2)	156	90	37 (41.1)
Thailand					
This study	400 (adult)	105 (26.2)	368	236	90 (38.1)
EUROPE					
Italy					
Falini B *et al.* (2005)[5]	591 (adult)	208 (35.2)	493	230	142 (61.7)
Cazzaniga G *et al.* (2005)[22]	107 (pediatric)	7 (6.5)	96	26	7 (26.9)
Roti G *et al.* (2006)[23]	120 (adult)	26 (21.7)	93	N/A	26 (N/A)
Germany					
Dohner K *et al.* (2005)[7]	300 (adult)	145 (48)	300	300	145 (48.3)
Schnittger S *et al.* (2005)[8]	401 (adult)	212 (52.9)	401	401	212 (52.9)
Thiede C *et al.* (2006)[9]	1485 (adult)	408 (27.5)	1395	709	324 (45.7)
Netherlands					
Verhaak R *et al.* (2005)[6]	275 (adult)	95 (34.5)	265	116	74 (63.8)

[a]*percentage of NPM1 mutations among all AML cases;* [b]*percentage of NPM1 mutations in cases with a normal karyotype; NA: not available.*

atric patients.[18] Furthermore, among adult patients, *NPM1* mutations were found more frequently in the older age group than the younger age group, as shown in Table 2, and this finding is supported by other studies.[7-10]

The effect of *NPM1* mutation in AML patients was found to be a favorable prognosis in most studies.[7-9] We could not observe a major difference in the overall survival (OS) in the Thai patients with and without NPM1 mutation (p=0.376). Interestingly, statistical analyses according to the combined *NPM1/FLT3* mutational status revealed a better outcome (p=0.036) for the *NPM1*-mutated/*FLT3* ITD–negative than *NPM1*$^+$/*FLT3-ITD*$^+$ and *NPM1-/FLT3-ITD*$^+$ subgroup, implicating the negative impact of *FLT3* mutations regardless of the *NPM1* mutational status. Nevertheless, because of the retrospective nature of this study, it might be useful to initiate a prospective clinical trial to confirm the true impact of *NPM1/FLT3* mutation on the clinical outcome of Southeast Asian patients.

Despite the fact that the fusion between the *NPM1* gene to its various partner genes had been known to be involved in hematologic malignancies for decades, the non-translocation mechanism of *NPM1* gene in AML, i.e. *NPM1* mutation, was only reported recently in 2005.[5-7] We report the first series of *NPM1* mutation from the ethnically distinct Southeast Asian region, representing the largest series in Asia (n=400). The incidence of *NPM1* mutation in Asian AML populations was previously known only from reports from China (n=28, 156),[19,20] Japan (n=257),[21] and Taiwan (n=173),[18] as summarized in Table 3. European reports were from Italy (n=107-2,562),[5,10,22,23] Germany (300-1,485),[7-9] and the Netherlands (n=275).[6] No AML studies concerning the *NPM1* mutations from other ethnic populations have been reported. Of all studies worldwide, *NPM1* mutations were mainly detected in AML patients, especially in adult AML patients with a normal karyotype (ranging from 40-60%). Only 2 cases of myelodysplastic syndrome from China[20] and 3 cases of chronic myelomonocytic leukemia from the United States were reported to have *NPM1* mutation type A.[24] The frequency of *NPM1* mutation in Thailand of 26.2% as reported by this study was comparable to that of Japan (24.9%), Taiwan (19.1%), Italy (21.7-35.2%) and Germany (27.5%) despite geographical and ethnic differences.

To summarize, *NPM1* mutations were most prevalent in Thai AML patients with a normal karyotype. Eight novel variants were identified and all the predicted mutated proteins gained the NES motif, supporting the aberrant process of NPM cytoplasmic localization associated with malignant transformation. Distinct prognostic subclasses of adult AML patients were identified based on the presence of *NPM1* and *FLT3* mutations. Targeting *FLT3* with specific *FLT3* inhibitors should provide benefits for the majority of *NPM1*-mutated AML patients who have coexistent unfavorable *FLT3* mutations.

Authorship and Disclosures

CUA was responsible for the initiation and execution of the entire project and writing of the manuscript. WT

supervised the molecular and data analysis and contributed to the revision of the manuscript. CB performed the experiments and data analysis and contributed to the drafting of the manuscript. We thank the staff of the Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital for excellent care of the patients in this study. CUA was the recipient of an award from the Anandamahidol Foundation and is the current principal investigator of the leukemia project funded by Mahidol University. CB is a graduate student whose thesis is partially supported by the Mahidol University Graduate Student Alumni Association. The authors reported no potential conflicts of interest.

References

1. Fröhling S, Scholl C, Gilliland DG, Levine RL. Genetics of myeloid malignancies: pathogenetic and clinical implications. J Clin Oncol 2005; 23:6285-95.
2. Grisendi S, Mecucci C, Falini B, Pandolfi PP. Nucleophosmin and cancer. Nat Rev Cancer 2006;6:493-505.
3. Frehlick LJ, Eirin-Lopez JM, Ausio J. New insights into the nucleophosmin/nucleoplasmin family of nuclear chaperones. Bioessays 2007; 29:49-59.
4. Naoe T, Suzuki T, Kiyoi H, Urano T. Nucleophosmin: a versatile molecule associated with hematological malignancies. Cancer Sci 2006;97: 963-9.
5. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 2005;352:254-66.
6. Verhaak RG, Goudswaard CS, van Putten W, Bijl MA, Sanders MA, Hugens W, et al. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood 2005; 106:3747-54.
7. Dohner K, Schlenk RF, Habdank M, Scholl C, Rucker FG, Corbacioglu A, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood 2005;106:3740-6.
8. Schnittger S, Schoch C, Kern W, Mecucci C, Tschulik C, Martelli MF, et al. Nucleophosmin gene mutations are predictors of favorable prognosis in acute myelogenous leukemia with a normal karyotype. Blood 2005;106:3733-9.
9. Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood 2006;107: 4011-20.
10. Falini B, Mecucci B, Saglio G, Lo Coco F, Diverio D, Brown P, et al. NPM1 mutations and cytoplasmic nucleophosmin are mutually exclusive of recurrent genetic abnormalities: a comparative analysis of 2562 patients with acute myeloid leukemia. Haematologica 2008;93: 439-42.
11. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. Blood 2008;111: 3941-67.
12. Mitelman F. ISCN: an international system for human cytogenetic nomenclature. Basel: S. Karker. 1995.
13. Auewarakul CU, Sritana N, Limwongse C, Thongnoppakhun W, Yenchitsomanus PT. Mutations of the FLT3 gene in adult acute myeloid leukemia: determination of incidence and identification of a novel mutation in a Thai population. Cancer Genet Cytogenet 2005; 162:127-34.
14. Auewarakul CU, Lauhakirti D, Tocharoentanaphol C. Frequency of RAS gene mutation and its cooperative genetic events in Southeast Asian adult acute myeloid leukemia. Eur J Haematol 2006;77:51-6.
15. Auewarakul CU, Leecharendkeat A, Tocharoentanaphol C, Promsuwicha O, Sritana N, Thongnoppakhun W. AML1 mutation and its coexistence with different transcription factor gene families in de novo acute myeloid leukemia (AML): redundancy or synergism. Haematologica 2007;92:861-2.
16. Falini B, Albiero E, Bolli N, De Marco MF, Madeo D, Martelli M, Nicoletti I, Rodeghiero F. Aberrant cytoplasmic expression of C-terminal-truncated NPM leukaemic mutant is dictated by tryptophans loss and a new NES motif. Leukemia 2007;2:2052-4.
17. Corbacioglu A, Frohling S, Paschka P, Marcucci G, Anhalt A, Urlbauer K, et al. Acute myeloid leukemia (AML) with 9q aberrations occurring within a non-complex karyotype is highly associated with CEBPA and NPM1 mutations - A joint analysis of the German-Austrian AML Study Group (AMLSG) and Cancer and Leukemia Group B (CALGB). ASH Annual Meeting Abstracts 2007;110:762.
18. Chou WC, Tang JL, Lin LI, Yao M, Tsay W, Chen CY, et al. Nucleophosmin mutations in de novo acute myeloid leukemia: the age-dependent incidences and the stability during disease evolution. Cancer Res 2006;66:3310-6.
19. Zhang Y, Zhang M, Yang L, Xiao Z. NPM1 mutations in myelodysplastic syndromes and acute myeloid leukemia with normal karyotype. Leuk Res 2007;31:109-11.
20. Yan L, Chen S, Liang J, Feng Y, Cen J, He J, et al. Analysis of NPM1 gene mutations in Chinese adults with acute myeloid leukemia. Int J Hematol 2007;86:143-6.
21. Suzuki T, Kiyoi H, Ozeki K, Tomita A, Yamaji S, Suzuki R, et al. Clinical characteristics and prognostic implications of NPM1 mutations in acute myeloid leukemia. Blood 2005;106: 2854-61.
22. Cazzaniga G, Dell'Oro MG, Mecucci C, Giarin E, Masetti R, Rossi V, et al. Nucleophosmin mutations in childhood acute myelogenous leukemia with normal karyotype. Blood 2005;106:1419-22.
23. Roti G, Rosati R, Bonasso R, Gorello P, Diverio D, Martelli MF, et al. Denaturing high-performance liquid chromatography: a valid approach for identifying NPM1 mutations in acute myeloid leukemia. J Mol Diagn 2006;8:254-9.
24. Caudill JS, Sternberg AJ, Li CY, Tefferi A, Lasho TL, Steensma DP. C-terminal nucleophosmin mutations are uncommon in chronic myeloid disorders. Br J Haematol 2006;133:638-41