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Cell line OCI/AML3 bears exon-12 *NPM* gene mutation-A and cytoplasmic expression of nucleophosmin

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We recently identified a new acute myeloid leukemia (AML) subtype characterized by mutations at exon-12 of the *nucleophosmin* (*NPM*) gene and aberrant cytoplasmic expression of NPM protein (NPMc+). NPMc+ AML accounts for about 35% of adult AML and it is associated with normal karyotype, wide morphological spectrum, CD34-negativity, high frequency of *FLT3-ITD* mutations and good response to induction therapy. In an attempt to identify a human cell line to serve as a model for the *in vitro* study of NPMc+ AML, we screened 79 myeloid cell lines for mutations at exon-12 of *NPM*. One of these cell lines, OCI/AML3, showed a TCTG duplication at exon-12 of *NPM*. This mutation corresponds to the type A, the *NPM* mutation most frequently observed in primary NPMc+ AML. OCI/AML3 cells also displayed typical phenotypic features of NPMc+ AML, that is, expression of macrophage markers and lack of CD34, and the immunocytochemical hallmark of this leukemia subtype, that is, the aberrant cytoplasmic expression of NPM. The OCI/AML3 cell line easily engrafts in NOD/SCID mice and maintains in the animals the typical features of NPMc+ AML, such as the NPM cytoplasmic expression. For all these reasons, the OCI/AML3 cell line represents a remarkable tool for biomolecular studies of NPMc+ AML.
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Introduction

Acute myeloid leukemia (AML) is clinically and molecularly heterogeneous.[1] About 40–50% of adult AMLs show normal karyotype[2] and their clinical and biological features are poorly understood.[1,3]

We identified mutations at exon-12 of the nucleophosmin (*NPM*) gene as the genetic lesion most specifically and frequently associated with normal karyotype in adult[4] and pediatric[5] AML. Named NPMc+ AML, this subgroup accounts for about 35% of adult AML (60% of all AML with normal karyotype), encompasses all FAB/WHO categories[6] except M3, M4eo and M7 and is characterized by multilineage involvement, CD34-negativity, high frequency of *FLT3* mutations and good response to induction therapy.[4]

Exon-12 *NPM* gene mutations are AML-specific since they are not detected in normal cells or other neoplasms. Frameshifts alter NPM protein at its C-terminus[4] and the mutated NPM protein localizes aberrantly in leukemic cell cytoplasm,[4] while wild-type NPM, a protein with multiple functional domains,[7] shuttles between nucleus and cytoplasm,[8] and is found mainly in the nucleolus.[9]

The mechanisms underlying abnormal cytoplasmic accumulation of mutant NPM protein and its interference with wild-type NPM, which is involved in ARF-p53 pathway regulation[10–12] and centrosome duplication,[13] remain to be clarified.

In an attempt to identify a human NPMc+ AML cell line, which would greatly help in addressing these issues and in investigating the activity of new drugs, we screened 79 myeloid cell lines for the presence of exon-12 *NPM* gene mutations and found one, OCI/AML3, exhibiting the typical molecular and biological features of NPMc+ AML.

Materials and methods

Mutational analysis of human myeloid cell lines

A total of 79 human myeloid cell lines were evaluated for *NPM* mutations (Table 1). Mutations at the exon-12 of the *NPM* gene are recurrent genetic aberrations in AML.[4] The *NPM* mutated and wild-type status of the 79 cell lines was verified by determining the size of the products of an *NPM* polymerase chain reaction (PCR) by capillary gel electrophoresis. *NPM* PCR analysis was performed with a DNA thermal cycler (Perkin Elmer Cetus, Heidelberg, Germany) under the following conditions: 30 s at 94°C for denaturation, 30 s at 53°C for annealing and 2 min at 72°C for extension. Genomic PCR amplification was performed using primer *NPM* intron 11 forward: 5′-TTA ACT CTC TGG TGG TAG AAT GAA-3′ and reverse-transcriptase PCR with primer *NPM* exon 11 forward: 5′-CAT CAA TTA TGT GAA GAA TTG CTT-3′. Reverse primer for both PCR analyses was *NPM* exon 12 reverse: 5′-TGT TAC AGA AAT GAA ATA AGA CGG-3′. The reverse primer was either labeled with the fluorescent dye D4 (for capillary electrophoresis) or not-labeled (for cloning experiments). Oligonucleotides were obtained from Proligo (Paris, France). For fragment size determination, 1 μl of the PCR products was combined with 0.25 μl of an internal size standard (size standard kit 400, Beckman-Coulter, Krefeld, Germany) in a total volume of 30 μl sample loading solution (SLS, Beckman-Coulter). Electrophoresis was carried out on the capillary electrophoresis system CEQ 8000 (Beckman-Coulter). The following injection and electrophoretic running conditions were used: 0.5 μl sample injection, 5 min DNA strand denaturation at 93°C, separation at 6000 V at 50°C and signal detection with calibrated D4 emission spectra. Fragment size determinations were performed using default fragment analysis parameters of the CEQ 8000 fragment analysis software, allowing the precise determination of fluorescent DNA fragments resulting in an electropherogram and a fragment summary list. *NPM*wt PCR products were 215 bp

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Table 1 Human myeloid cell lines screened for exon-12 *NPM* gene mutations

Cell line	*FAB*	*DSM ACC*
AML-14	M2	
AML-193	M5	549
AS-E2	M6	
CHRF-288-11	M7	
CMK	M7	392
CMY	M7	
CTS	M1	
CTV-1	M5	40
ELF-153	M7	
EOL-1	eosino	386
F-36P	M6	543
FKH-1	M4	
GDM-1	M4	87
GF-D8	M1	
HEL	M6	11
HL-60	M2	3
HNT-34	M4	
HT-93A	M3	
HU-3	M7	
IMS-M1	M5	
K-051	M2	
KASUMI-1	M2	220
KASUMI-3	M0	
KBM-3	M4	
KG-1	NI	14
KMOE-2	M6	37
KP-MO-TS	M5	
KY-821	M2	
M-07e	M7	104
MARIMO	M2	
MB-02	M7	
ME-1	M4	537
MEGAL	M7	
MKPL-1	M7	
ML-2	M4	15
MML-1	M1	
M-MOK	M7	
MOLM-13	M5	554
MOLM-16	M0	555
MOLM-17	sAML	
MONO-7	M0	
MONO-MAC-6	M5	124
MR-87	NI	
MUTZ-2	M2	271
MUTZ-3	M4	295
MUTZ-8	M4	
MUTZ-11	M4	
MV4;11	M5	102
NB-4	M3	207
NOMO-1	M5	542
OCI-AML-1	M4	
OCI-AML-2	M4	99
OCI-AML-3	M4	582
OCI-AML-4	M4	
OCI-AML-5	M4	247
OCI-AML-6	M4	
OCI-M1	M6	529
OCI-M2	M6	
OHN-GM	tAML	
OMA-AML-1	M4	
PL-21	M3	536
SAML-2	tAML	
SIG-M5	M5	468
SKM-1	M5	547
SKNO-1	M2	
TF-1	M6	334
THP-1	M5	16
TK-1B	M4	
TMD-7	M7	
TSU-1621MT	M4	
U-937	M5	5
UCSD/AML1	NI	
UF-1	M3	
UG-3	M5	
UOC-M1	M1	
UT-7	M7	137
YK-M2	M5	
YNH-1	M1	
X-376	NI	

DSM ACC, cell line available from DSMZ cell lines bank (www.dsmz.de); FAB, French–American–British classification system; NI, not indicated; sAML, secondary AML; tAML, therapy-related AML.

(genomic PCR) and 139 bp (RT-PCR). *NPM*m primary AML cells, used as positive control, exhibited 4 bp larger signals in addition to the *NPM*wt signals. For the identification of *NPM* variants in cell lines, a second PCR analysis was performed, this time with unlabeled oligonucleotides. The PCR products were cloned into the pGEM-T Easy Vector System (Promega, Mannheim, Germany), *NPM*m and wt clones were identified with the described detection system and positively identified plasmids were sequenced (MWG Biotech, Ebersberg, Germany).

Cytogenetic analysis

Karyotypic analysis and fluorescence *in situ* hybridization (FISH) were performed as described previously[14]

Antibodies

Monoclonal anti-NPM and anti-CD68 antibodies have been previously described.[9,15,16] An affinity-purified rabbit polyclonal antibody (SIL-A) was generated against a synthetic 18mer peptide (NH_2-QEAIQDLCLAVEEVSLRK-COOH) (Primm srl, Milan, Italy), which recognizes the NPM mutant A^4 but not wild-type NPM protein. Monoclonal antibody against nucleolin (C23) was purchased from Dako Cytomation (Glostrup, Denmark).

Engraftment in NOD/SCID mice

NOD/SCID mice were purchased from Charles River Italia Spa (Calco, Italy) and maintained under sterile conditions in the animal facilities at the University of Perugia, Perugia, Italy. Six mice were injected subcutaneously with OCI/AML3 cells at dosage ranging from 20×10^6 to 50×10^6 OCI/AML3 cells.

Immunocytochemical detection of NPM

For immuno-alkaline phosphatase (APAAP) detection of wild-type and mutant NPM proteins, we used cytocentrifuge preparations of acetone- or 4% paraformaldeyde-fixed (10 min) OCI/AML3 cells, and paraffin sections (3 μm) from B5-fixed cell line pellets (each containing 10^7 cells). Paraffin sections were subjected to antigen retrieval by microwaving in 0.1 mM EDTA pH 8.0.[4] The immuno-alkaline phosphatase APAAP technique was used for immunostaining.[17]

For immunofluorescence, paraffin sections of OCI/AML3 cells were incubated with the anti-NPM monoclonal antibody[9,15] followed by a secondary goat anti-mouse antibody conjugated with Alexa 488 (Molecular Probes, OR, USA), counterstained with propidium iodide and analyzed with a confocal microscope (Zeiss, LSM 510). Images were collected with a Zeiss LSM 510 confocal microscope Carl Zeiss, Jena, DE, Germany) using the 488 nm (for Alexa 488) and the 543 nm (for propidium iodide) laser lines for excitation. AOTF-controlled tuning of laser lines, diameters of pinholes and light collection configuration were optimized to obtain the best signal-to-noise ratio and to avoid any fluorescence crossover.

The LSM 510 software was used for microscope regulation and collection of images. The images were transferred to an SGI Octane workstation (Silicon Graphics, Mountain View, CA, USA) for further processing. Three-dimensional reconstruction of the slices was performed with the shadow technique, using the Imaris (Bitplane, Zurich, CH) software.

Western blotting

Nuclear and cytoplasmic extracts were prepared using a slightly modified Schreiber method.[18] Briefly, 5×10^6 cells were collected, washed with phosphate-buffered saline and used either fresh or after being snap frozen in liquid nitrogen. Either cells or frozen cell pellets were resuspended in 150 μl of buffer A containing 10 mmol/l HEPES (pH 7.9), 1.5 mmol/l $MgCl_2$, 10 mmol/l KCl, 0.2 mmol/l EDTA, 1 mmol/l dithiothreitol and proteases inhibitors (2 μg/ml aprotinin, 2 μg/ml leupeptin, 1 mM phenylmethane sulfonyl fluoride, 1 mM sodium orthovanadate) and incubated for 15 min on ice, after which 7.5 μl of a 10% solution of Nonidet P-40 (0.5%, final) was added and the tube was vigorously vortexed for 10 s. The homogenate was centrifuged at 12 000 r.p.m for 30 s. The cytoplasmic supernatant fraction was spun once more to ensure complete removal of nuclei. The nuclear pellet was washed again with buffer A with centrifugation as above. The nuclear pellet was then resuspended in 50 μl of lysis buffer C containing 10 mmol/l HEPES (pH 7.9), 0.42 mol/l NaCl, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l EDTA, 0.5 mmol/l dithiothreitol, 25% glycerol and proteases inhibitors. After incubation on ice for 15 min (with occasional vortexing) the samples were centrifuged at 12 000 r.p.m for 15 min at 4°C. The supernatant was recovered as nuclear extracts. Nuclear and cytoplasmic extracts were snap frozen in liquid nitrogen and stored at −80°C until use.

Whole cell lysates (WCL) were prepared by addition of ice-cold lysis buffer (1% NP-40, 150 mmol/l NaCl, 25 mmol/l Tris pH 7.5, 1 mmol/l EDTA, 1 mmol/l Na_3VO_4, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 mmol/l phenylmethylsulfonyl fluoride) on ice for 20 min. Cell lysates were clarified by centrifugation at 14 000 *g* for 10 min at 4°C. Proteins were boiled in SDS sample buffer, separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene difluoride (PVDF; Millipore, Bedford, MA, USA) and probed with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies. Polypeptides recognized in the Western blot were detected using the enhanced chemiluminescence (ECL) methods according to the manufacturer's instructions (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA).

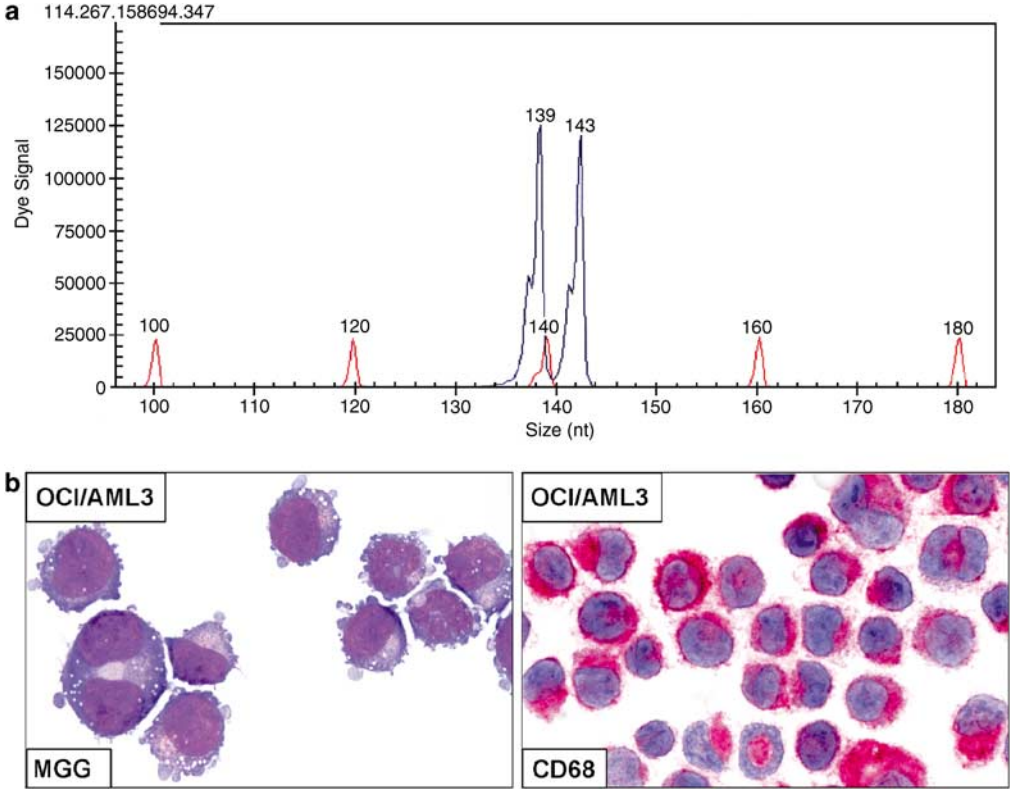

Figure 1 The OCI/AML3 cell line carries mutation A at exon-12 of the *NPM* gene. (a) Electropherogram of *NPM* RT-PCR of cell line OCI/AML3. Cell line OCI/AML3 expresses wild-type (139 bp) and mutant (143 bp) version of the *NPM* gene. (b) (left) Morphological appearance of OCI/AML3 cells (May Grünwald Giemsa; ×1000); (right) the OCI/AML3 cells express the macrophage-restricted CD68 (monoclonal antibody PG-M1; APAAP technique; ×1000).

Results

OCI/AML3 cell line bears mutation A at exon-12 of the NPM *gene*

OCI/AML3 was the only myeloid cell line among the 79 tested that expressed a signal in addition to and 4 bp larger than the wild-type *NPM* signal (Figure 1a). Sequencing of cloned *NPM*-mutated PCR product showed TCTG duplication at positions 956–959 of exon-12 of the reference sequence (GenBank accession number NPM-002520). This mutation was heterozygous and corresponded to the type A we previously described in 77% of primary NPMc+ AMLs.[4] Exon-12 *NPM* mutation of type A causes a frameshift in the region encoding the C-terminal of the NPM protein. As a consequence, the last seven amino acids (WQWRKSL) of NPM are replaced by 11 different residues (CLAVEEVSLRK). Important changes at the C-terminus of NPM mutant A include mutations of both tryptophans at position 288 and 290 which are important for nucleolar localization[19] and the creation of an NES motif.[20]

Phenotypic and cytogenetic/FISH features of the OCI/AML3 cell line

The OCI/AML3 cell line was established from the peripheral blood taken from a 57-year-old male with AML FAB-M4.[21] The OCI/AML3 cells show a myeloid morphology (Figure 1b, left-hand side) and the immunophenotype: $CD3^-$, $CD4^+$, $CD8^-$, $CD10^-$, $CD13^+$, $CD14^-$, $CD15^+$, $CD19^-$, $CD30^-$, $CD34^-$, $CD41^+$, $CD42b^-$, $CD68^+$ (Figure 1b, right-hand side), $CD117^-$ and $CD235a^+$.

Karyotype of the AML at diagnosis from which OCI/AML3 was established was not available. The consensus karyotype was 48(45-50)<2n>X/XY, +1, +5, +8, der(1)t(1;18) (p11;q11),i(5p), del(13)(q13q21),dup(17)(q21q25);sdl with r(Y)x1-2;hemizygous for RB1 (Figure 2).

FISH analysis of OCI/AML3 revealed no structural rearrangements involving *MLL*, *MOZ*, or *CBFB*. *FLT3* internal tandem duplication (ITD) is a recurrent aberration in AML,[22] but quite rare in cell lines.[23] Although *FTL3* ITD are twice as frequent in NPMc+ than in NPMc− AML with normal karyotype,[4] the cell line OCI/AML3 exhibits neither *FLT3* ITD nor *FLT3* D835 point mutation.[23]

OCI/AML3 cell line exhibits aberrant cytoplasmic NPM expression

Immunostaining with specific monoclonal antibodies[9] detected NPM both in the cytoplasm and nucleus (nucleoli) of OCI/AML3 cells (Figure 3a, top left, middle left). This contrasts with the nucleus-restricted expression of NPM observed in AMLs bearing wild-type *NPM*, such as the cell line HL-60 (Figure 3a, top right). Therefore, the NPM staining pattern in the OCI/AML3 cell line is identical to that observed in primary NPMc+ AML samples.[4]

However, anti-NPM monoclonal antibodies do not distinguish between wild-type and mutated NPM which are both

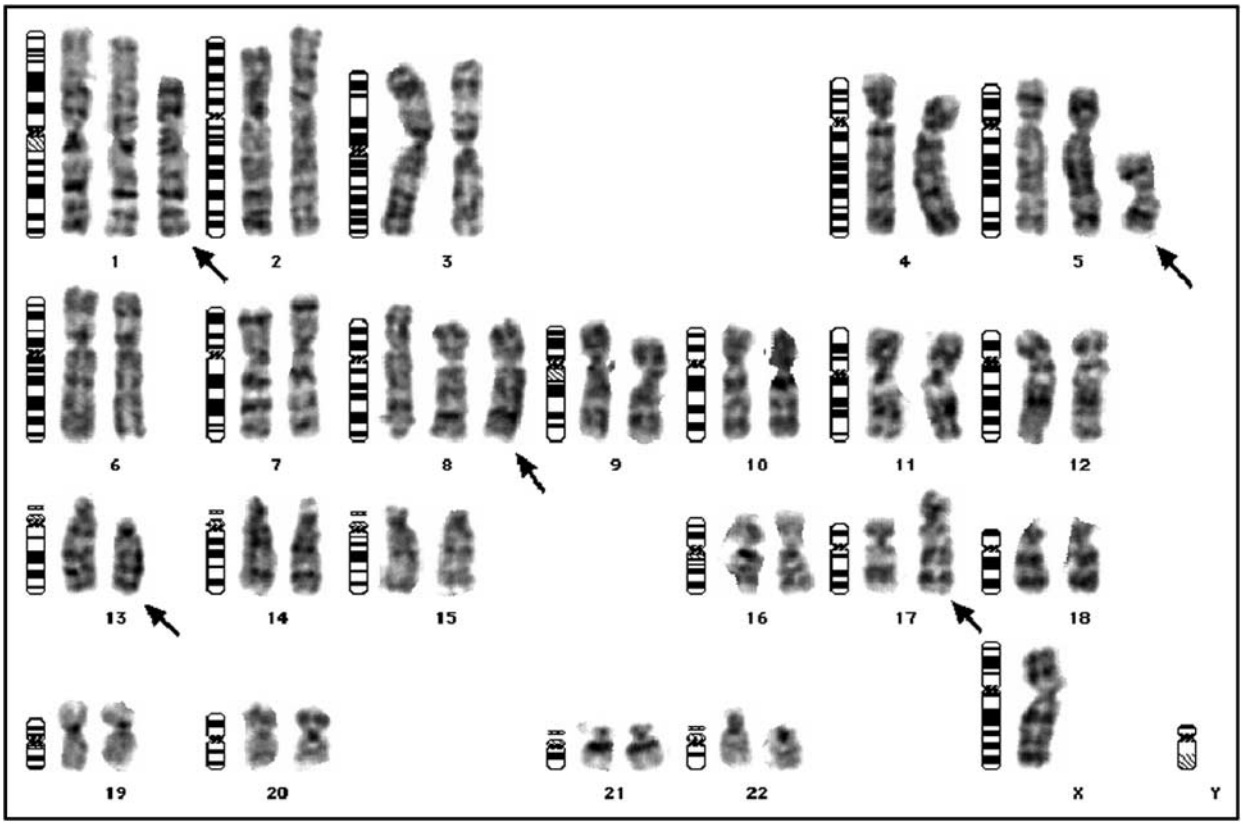

Figure 2 Karyotype of the OCI/AML3 cell line. Representative metaphase cell harvested from OCI/AML3 cultures. Note the presence of an additional copy of an isochromosome for the short arms of chromosome 5 and trisomy 8, together with a partial trisomy of chromosome 1 as described for a distinct subgroup of AML patients.[25] Abnormal chromosomes are indicated by arrows. Rearrangements were confirmed by locus-specific FISH.

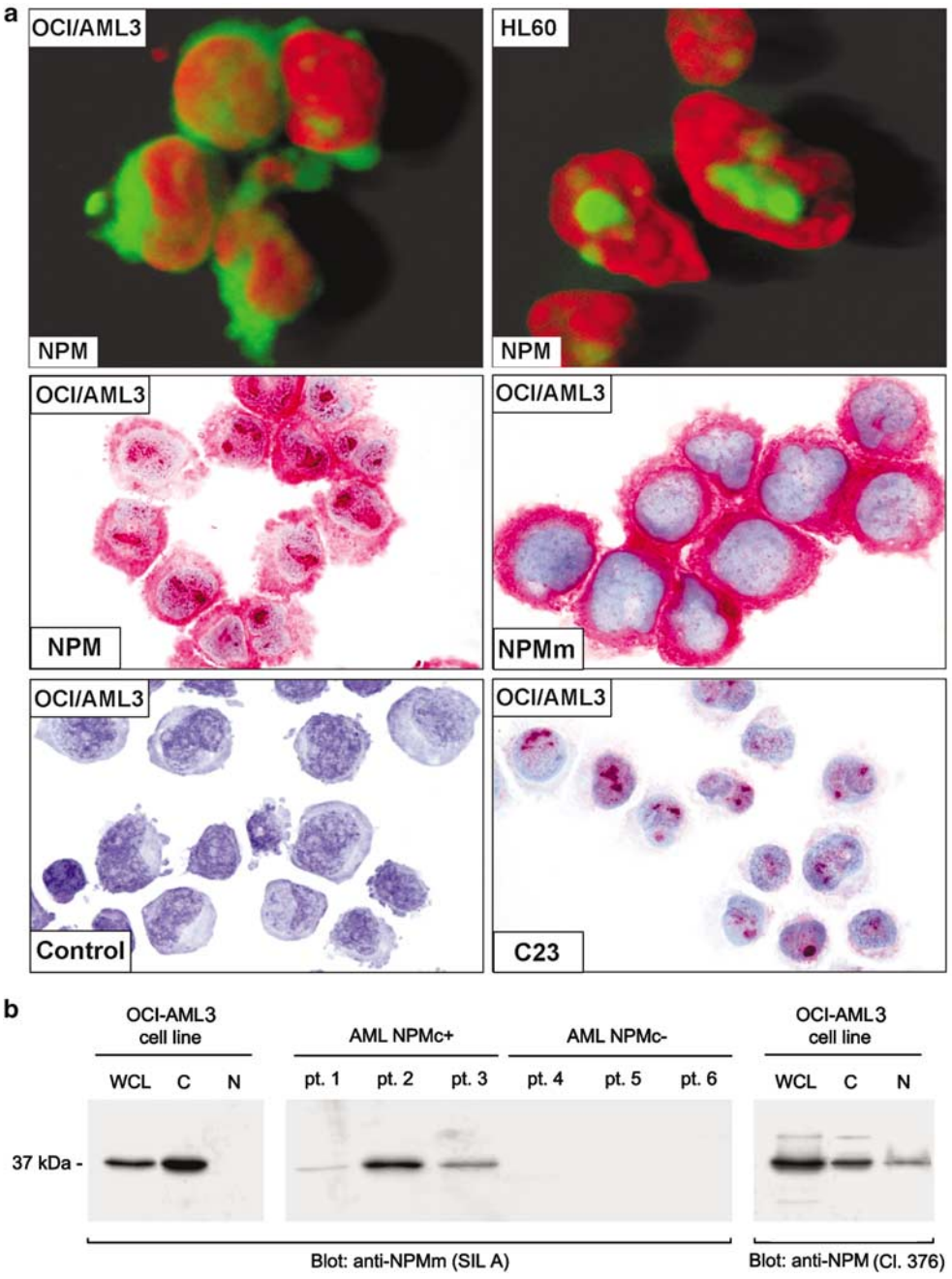

Figure 3 The NPM mutant protein A shows a cytoplasmic-restricted expression in OCI/AML3 cells. (a) (Top left) Immunofluorescence analysis with an anti-NPM monoclonal antibody shows NPM in the cytoplasm and nucleoli of OCI/AML3 cells (nuclei are counterstained in red with propidium iodide). (Top right) The same antibody recognizes NPM exclusively in the nucleoli of HL-60 cells that bear only wild-type NPM. (Middle left) Monoclonal antibody anti-NPM labels the wild-type NPM protein in the nucleus and the mutated NPM protein in the cytoplasm of the OCI/AML3 cells. (Middle right) The SIL-A antibody identifies the NPM mutant A (NPMm) as exclusively expressed in the cytoplasm of the OCI/AML3 cells. (Bottom left) Incubation of the SIL-A antibody with the 18mer immunogen peptide abolishes the cytoplasmic reactivity confirming the specificity of the antibody. (Bottom right) Expression of nucleolin (C23) is restricted to the nucleus (mainly nucleoli) of OCI/AML3 cells. (b) Western blotting: The antibody specific for the mutated NPM protein (anti-NPMm, SIL-A) identifies a band of the expected molecular weight of NPM in the cytoplasmic (C) but not in the nuclear (N) fractions of the OCI/AML3 cells (left panel). In contrast, the anti-NPM monoclonal antibody 376 (which cannot discriminate between wild-type and mutated NPM) recognizes the 37 kDa band both in the cytoplasmic and nuclear fraction of the OCI/AML3 cells (right panel). The anti-NPMm, SIL-A antibody identifies the 37 kDa NPM band in whole lysates of NPMc+ AML patients 1–3 bearing exon-12 *NPM* mutation but not in NPMc− AML patients 5–6 bearing wild-type NPM (central panel). WCL indicates: whole-cell lysates. NPMc+ indicates: NPM-cytoplasmic positive; NPMc− indicates: NPM-cytoplasmic negative.

present in leukemic cells due to the fact that exon-12 *NPM* mutations are consistently heterozygous.[4] In order to investigate the subcellular expression of the mutant A without the interference of the NPM wild-type protein, we stained the OCI/AML3 cells with an antibody specific for mutant A and found that mutated NPM is expressed exclusively in the cytoplasm (Figure 3a, middle right); pre-incubation with the immunogen peptide abolished the reactivity of the anti-NPM-mutated

antibody (Figure 3a, bottom left). In contrast, the anti-nucleolin antibody only labeled nucleoli (Figure 3a, bottom right). Immunohistochemical findings were confirmed by Western blotting analysis (Figure 3b).

Alltogether, these findings demonstrate that OCI/AML3 cells bear the hallmark of primary NPMc+ AML, that is mislocalization of the mutant NPM protein in the cytoplasm.

Engraftment in NOD/SCID mice

Subcutaneous injection of OCI/AML3 cells in NOD/SCID produced palpable tumors in all six injected animals by days 25–30. At post-mortem examination, mice showed disseminated disease that was confirmed by morphological and immunohistochemical studies showing the typical aberrant expression of NPM in the cytoplasm of the leukemic cells (Figure 4). Since the anti-NPM monoclonal antibodies recognize both human and mouse NPM, nucleus-restricted expression of murine NPM served as a control.

Discussion

Human cell lines derived from AML bearing recurrent chromosomal abnormalities represent remarkable tools for biological and molecular studies of these diseases.[14]

We recently identified a new AML subtype characterized by mutations at exon-12 of the *NPM* gene and aberrant cytoplasmic expression of the NPM protein (NPMc+).[4] NPMc+ AML accounts for about 35% of adult AML and it is characteristically associated with a normal karyotype, wide morphological

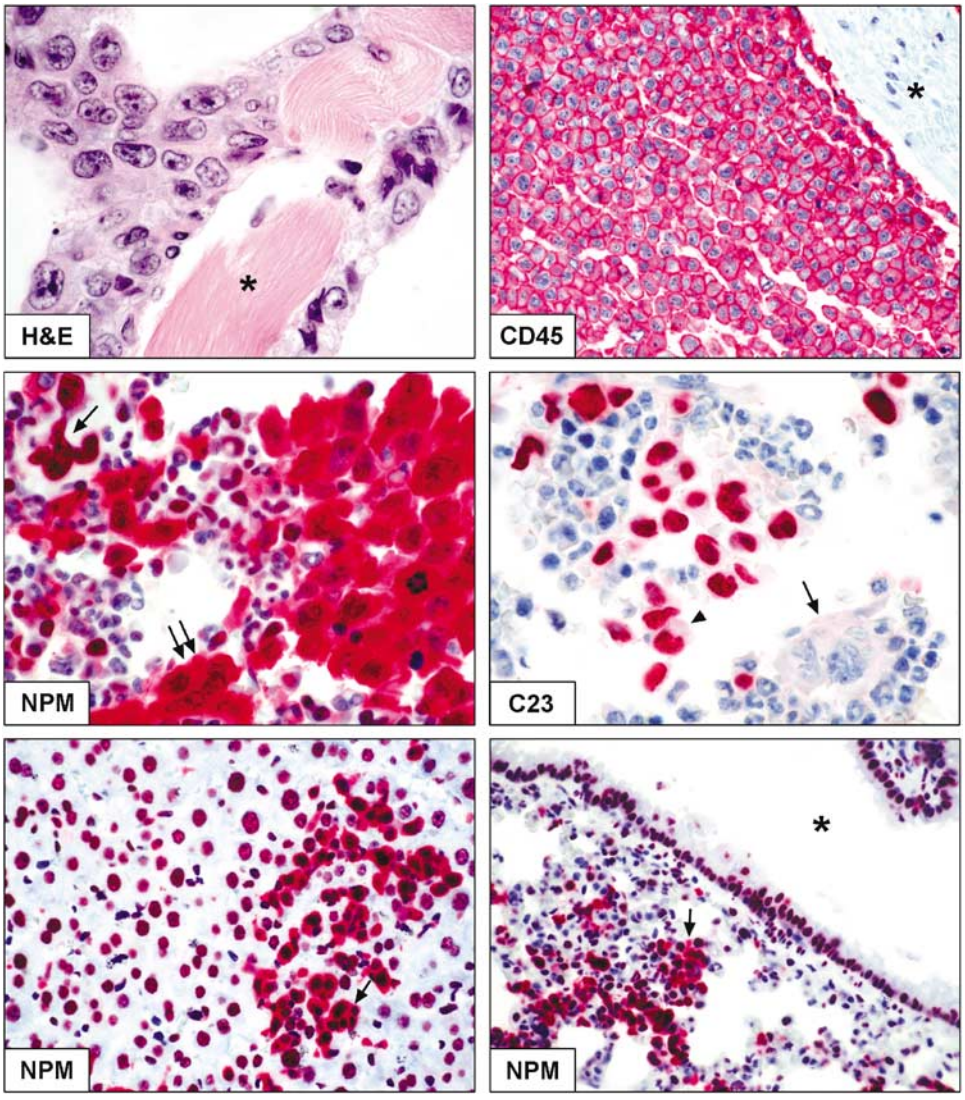

Figure 4 Engrafment of the OCI/AML3 cells in NOD/SCID mice. (Top left) Infiltration of muscle (asterisk) by OCI/AML3 cells (Hematoxylin–Eosin; ×800); (top right) OCI/AML3 cells infiltrating the muscle (asterisk) express the human CD45 antigen (×400); (middle left) bone marrow involvement by OCI/AML3 cells expressing cytoplasmic nucleophosmin (double arrow); the single arrow points to a residual mouse megakaryocyte showing the expected nucleus-restricted expression of NPM (×800); (middle right) OCI/AML3 cells infiltrating the marrow show nucleus-restricted expression of nucleolin/C23 (arrowhead); a residual mouse megakaryocyte (arrow) is not labeled by the anti-human nucleolin (C23) monoclonal antibody (×800); (bottom left) liver infiltration by OCI/AML3 expressing cytoplasmic NPM (arrow) (×400); (bottom right) lung infiltration by OCI/AML3 with cytoplasmic NPM (arrow); the asterix indicates a bronchiolar lumen (×400). All immunostainings were performed using APAAP technique.

spectrum, CD34 negativity, high frequency of *FLT3-ITD* mutations and a relatively good response to induction therapy.[4] Our numerous attempts to establish a cell line from these leukemic patients were unsuccessful. This prompted us to carry out a mutational screening for exon-12 *NPM* mutations in a large number of available human myeloid cell lines.

Here, we report that the OCI/AML3 cell line bears the characteristic molecular and biological features of the NPMc+ AML. The OCI/AML3 cell line was the only human myeloid cell line, among the 79 tested, to bear mutations at the exon-12 of the *NPM* gene. As *NPM* mutations occur in about 35% of adult AML patients,[4] their low frequency in myeloid cell lines is somewhat surprising, but may indicate this leukemia subtype is difficult to grow in culture, possibly because of its dependence on host growth factors. Notably, the OCI/AML3 cell line bears mutation A, which is the most frequent type of mutations occurring at exon-12 of the *NPM* gene in AML (about 80% of cases).[4] In this regard, OCI/AML3 is a model representative of most patients with NPMc+ AML.

The immunophenotype of the OCI/AML3 cell line is also consistent with a derivation from a patient with NPMc+ AML. The OCI/AML3 cells express macrophage markers such as CD68 (PG-M1)[16] and lack of CD34. This is in keeping with the finding that primary NPMc+ AML are most frequently of the M4 and M5 type and show absence of CD34 both at immunohistochemistry[4] and gene expression profiling.[24]

Mutations at exon-12 of the *NPM* gene are the genetic lesions most specifically and frequently associated with normal karyotype in adult AML.[4] NPMc+ AML account for about 60% of all adult AML with a normal karyotype. Karyotype of patient primary AML cells from which the OCI/AML3 was established was not known. The results of our cytogenetic investigations deserve some comments. Trisomy 8 occurs in 10–15% of AML in all FAB subgroups, alone or together with simple karyopypic changes.[22] Interestingly, +8 partners i(5p) in a subset of AML-M5 patients, possibly associated with 1q trisomy[25] as found in OCI/AML3. This is in keeping with the finding that NPMc+ AML are more frequent in M5 than other FAB subtypes.[4] Deletion of proximal 13q (including the *FLT3* locus at 13q13) as present in OCI/AML3 may also partner +8 in AML patients possibly targeting loss of heterozygosity affecting the RB1 locus at 13q14.[26] The association in OCI/AML3 of the above chromosomal abnormalities and *NPM* gene mutation is not surprising since we previously reported that about 14% of NPMc+ AML bear nonmajor chromosomal abnormalities.[4]

The OCI/AML3 displays one of the most remarkable features of NPMc+ AML, that is, the dislocation of the NPM protein in the cytoplasm. According to our results, this finding is certainly due to the presence in the cytoplasm of the NPM mutant A and, possibly, of the wild-type NPM protein that could be aberrantly recruited in the cytoplasm through formation of heterodimers with the mutant.[27] In contrast, we regard nuclear labeling of OCI/AML3 for NPM to be exclusively due to the presence in that site of the wild-type NPM protein, as proved by the finding that an antibody specifically directed against the mutant only detects the abnormal NPM protein in the cytoplasm. Mechanism of altered-nucleo-cytoplasmic traffic of NPM in leukemic cells is unknown and the OCI/AML3 represents a good *in vitro* model for addressing this issue and analyzing the drug's ability to interfere with this process. Moreover, OCI/AML3 cells easily engraft in NOD/SCID mice and this may serve as a pre-clinical model for testing new therapeutic modalities.

In conclusion, the OCI/AML3 represents the only permanent cell line sharing all molecular and biological features of primary NPMc+ AML and emerges as a remarkable tool for studying this new leukemia subtype.

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