ARTICLE
Journal of Cellular Biochemistry 111:67–74 (2010)

Journal of Cellular Biochemistry

Localization of Nucleophosmin in Nuclear Matrix and Changes in Its Expression During the Differentiation of Human Neuroblastoma Induced by Retinoic Acid

Song-Lin Shi,[1,2] Ying Liang,[1] Qi-Fu Li,[1*] Qing-Rong Liu,[3] Guang-Jun Jing,[1] San-Ying Wang,[1] Xiu-Yan Zhang,[1] and Fu-Yun Wu[1]

[1] *The Key Laboratory of Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Science, Xiamen University, Xiamen 361005, PR China*

[2] *Medical College of Xiamen University, Xiamen 361005, PR China*

[3] *Molecular Neurobiology Branch, National Institute on Drug Abuse-Intramural Research Program (NIDA-IRP), NIH, Department of Health and Human Services (DHSS), 333 Cassell Drive, Baltimore, Maryland 21224*

ABSTRACT

In this article, we selectively extracted the nuclear matrix and intermediate filament system of human neuroblastoma SK-N-SH cells pre- and post-treated with retinoic acid (RA). The distribution of nucleophosmin (NPM) in the nuclear matrix and its colocalization with several products of related genes were investigated. Results from two-dimensional gel electrophoresis and MALDI-TOF showed that NPM was a component of the nuclear matrix and its expression in SK-N-SH cells post-treated with RA was down-regulated. Immunofluorescent microscopy observations further showed that NPM was localized in the nuclear matrix of SK-N-SH cells, and its expression level and distribution were altered after treatment with RA. The colocalization of NPM with c-myc, c-fos, p53, and Rb in SK-N-SH cells was observed under a laser scanning confocal microscope, but the colocalization region was changed by RA. Our results prove that NPM is a nuclear matrix protein, which is localized in nuclear matrix fibers. The colocalization of NPM with its related genes and oncogenes affect the differentiation of SK-N-SH cells. The expression of NPM and its distribution in the process of cell differentiation deserve more intensive investigation. J. Cell. Biochem. 111: 67–74, 2010.

KEY WORDS: NUCLEOPHOSMIN; NUCLEAR MATRIX; HUMAN NEUROBLASTOMA; CELL DIFFERENTIATION

Nucleophosmin, also known as NPM, B23, Numatrin, and NO38, is the major nucleolar phosphoprotein involved in multiple cellular functions. It is closely related to the assembly and synthesis of RNA and the replication of chromosomes, and also acts as a molecular chaperone that prevents protein accumulation. It can regulate the activity of p53 and p14, both of which are tumor suppressor genes. It inspects the activity of nucleolus and cell proliferation [Ye, 2005; Naoe et al., 2006; Frehlick et al., 2007]. Research data show that NPM is a potent oncogene and was usually over-expressed in a number of tumor cell lines. Its expression was further down-regulated during the induced differentiation of tumor cells [Yun et al., 2003; Lim and Wang, 2006]. As a nucleo-cytoplasm shuttle protein, NPM can maintain nuclear stability and regulate gene expression. However, its intracellular localization and function in the malignant phenotype reverse are still not clear. In recent years, our research group used HMBA, retinoic acid (RA), and RCT to induce human osteosarcoma MG-63 cells and human hepatocarcinoma SMMC-7721 cells into terminal differentiation. We found that the localization of NPM in the nuclear matrix-intermediate filament (NM-IF) system was altered, which revealed that NPM was closely related to the differentiation of tumor cells [Li et al., 2008a,b].

In this article, RA was used to induce human neuroblastoma SK-N-SH cells. The localization and expression of NPM in the nuclear matrix and its relationship with the oncogene and tumor suppressor gene were further studied. Our results may help reveal the regulatory role of NPM in tumor cell differentiation, as well as provide scientific basis for the mechanism of neuroblastoma carcinogenesis and reversion.

Song-Lin Shi and Ying Liang contributed equally to this work.

Grant sponsor: National Natural Science Foundation of China; Grant number: 30871241.

*Correspondence to: Prof. Qi-Fu Li, The Key Laboratory of Education Ministry for Cell Biology and Tumor Cell Engineering, School of Life Sciences, Xiamen University, Xiamen 361005, Fujian Province, PR China. E-mail: chifulee@xmu.edu.cn

Received 20 December 2009; Accepted 12 April 2010 • DOI 10.1002/jcb.22663 • © 2010 Wiley-Liss, Inc.
Published online 23 April 2010 in Wiley Online Library (wileyonlinelibrary.com).

MATERIALS AND METHODS

CELL CULTURE AND TREATMENT

Human neuroblastoma SK-N-SH cells, provided by the China Center for Type Culture Collection, were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μg/ml kanamycin at 37°C with 5% CO_2 in air. Twenty-four hours after seeding onto dishes, SK-N-SH cells were maintained in a culture medium containing 1 μM RA (Sigma) for 7 days to induce differentiation. Meanwhile, another set of SK-N-SH cells were cultured in RPMI-1640 medium as a control.

SELECTIVE EXTRACTION OF NUCLEAR MATRIX PROTEINS (NMPs)

The selective extraction method we used was optimized according to the manner described by Michishita et al. [2002]. SK-N-SH cells were rinsed twice with ice-cold PBS and then extracted by a cytoskeleton (CSK100) buffer (100 mM NaCl, 3 mM $MgCl_2$, 10 mM PIPES, 300 mM sucrose, 0.5% Triton X-100, 1 mM EGTA, and 1 mM PMSF, pH 6.8) for 10 min at 0°C. After centrifugation at 1,000*g* for 5 min, the pellets were rinsed with ice-cold PBS to remove the soluble cytoplasmic proteins and then recentrifuged and suspended within the digestion buffer CSK50 (identical to CSK100 buffer, except with 50 mM NaCl instead of KCl) containing 400 mg/ml DNase for 30 min at room temperature. Cold ammonium sulfate at a final concentration of 0.25 M was added to terminate enzyme digestion.

After centrifugation at 1,000*g* for 5 min, the pellets were rinsed with CSK50 buffer and then dissolved in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 1.5% Triton X-100, 1% Pharmalyte (pH 3-10; Amersham Biosciences), 65 mM DTT, 40 mM Tris, 5 mg/ml aprotinin, 1 mg/ml leupeptin, 1 mg/ml pepstatin, 2 mM PMSF, and 4 mM EDTA). The sample was sonicated at 0°C for 30 min and centrifuged at 10,000*g* for 1 h. The protein concentrations of the control and treated supernatants were determined by the Bradford assay, diluted to the same concentration with lysis buffer, and stored at −80°C.

TWO-DIMENSIONAL (2D) GEL ELECTROPHORESIS AND PROFILE ANALYSIS

2D PAGE was performed using standard methods. The gels were stained using a silver nitrate protocol compatible with mass spectrometry. Image scanning (UMAX Power Look III) and analysis (PD Quest 8.0 software; Bio-Rad) of the triplicate sets of silver-stained 2D gels were performed. The intensity of each protein spot was normalized to the total intensity of the entire gel. The spots of protein with at least twofold change in intensity were defined as differentially expressed NPMs.

MALDI-TOF-MS ANALYSIS AND NPM IDENTIFICATION

Spots representing the differentially expressed proteins were cut from the gels. After a series of steps including silver removal, reduction with DL-dithiothreitol, alkylation with iodacetamide, and inner-gel digestion with trypsin, peptide mass fingerprints (PMFs) were generated using a Bruker III MALDI-TOF mass spectrometer. Flex Analysis software was used to analyze the PMF data in order to calibrate and remove polluted peaks. Data were searched against the NCBInr and Swiss-Prot protein databases using the Mascot tool from Matrix Science.

WESTERN BLOTTING

The proteins were separated electrophoretically in 12% polyacrylamide gels and transferred onto PVDF membranes. The membranes were blocked for 1.5 h at room temperature in Tris-buffered saline-Tween-20 (TBST) (20 mM Tris–HCl, pH 7.4, and 150 mM NaCl with 0.05% Tween-20) containing 5% nonfat milk powder and incubated overnight at 4°C with mouse NPM monoclonal antibody (1:400, Abcam) in TBST. The membranes were then washed four times in TBST for 40 min, incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (1:10,000; Santa Cruz Biotechnology) for 40 min, and washed three times in TBST. Immunoreactive bands were revealed using an enhanced chemiluminescence detection system (Pierce). Two percent BSA was used in the negative group instead of the primary antibodies. Beta-actin was also detected as an internal control.

SAMPLE PREPARATION FOR IMMUNOFLUORESCENT MICROSCOPE

After pre-treatment with 1 μmol/L RA for 5 days, SK-N-SH cells were seeded onto cover slips for another 2–3 days. SK-N-SH cell was selective extracted according to the method described by Li [1999], and the NM-IF system on the cover slips was fixed with paraformaldehyde for 10 min. Non-specific reactivity was blocked by incubating the membranes at room temperature for 1 h in 3% BSA. The cover slip was incubated overnight with primary antibody against NPM at 4°C. After washing, the secondary antibody (labeled with the fluorescent dye tetramethylrhodamine isothiocyanate (TRITC)) was added onto the cover slip for 30 min at 37°C in the dark. The cover slip was sealed with an anti-fluorescence quencher. Three percent BSA was used in the negative group instead of the primary antibody.

SAMPLE PREPARATION FOR LASER SCANNING CONFOCAL MICROSCOPY

The cover slip was washed twice with PBS and immersed in 0.5% Triton X-100 for 5–10 min, fixed with 4% paraformaldehyde for 10 min, and blocked with 3% BSA for 1 h. The cover slips were incubated with different primary antibody combinations (NPM/c-myc, NPM/c-fos, NPM/p53, and NPM/Rb) at 4°C overnight. After washing, the cover slips were incubated with secondary antibodies (labeled with fluorescein) at 4°C for 3 h. After sealing with an anti-fluorescence quencher, the cover slip was observed under a laser scanning confocal microscope (TCS-SP2 MP). Three percent BSA was used in the negative group instead of the primary antibody.

RESULTS

NPM DOWN-REGULATION AS SHOWN BY 2D PAGE AND IMAGE ANALYSIS

The nuclear matrix proteins extracted from the control and RA-treated groups of human neuroblastoma SK-N-SH cells were subjected to 2D PAGE in triplicates. Quantification of images of silver-stained 2D gels was performed using PD Quest 8.0 software (Bio-Raid). Figure 1A shows the differentially expressed protein spots in the control and treated groups, Figure 1B shows the

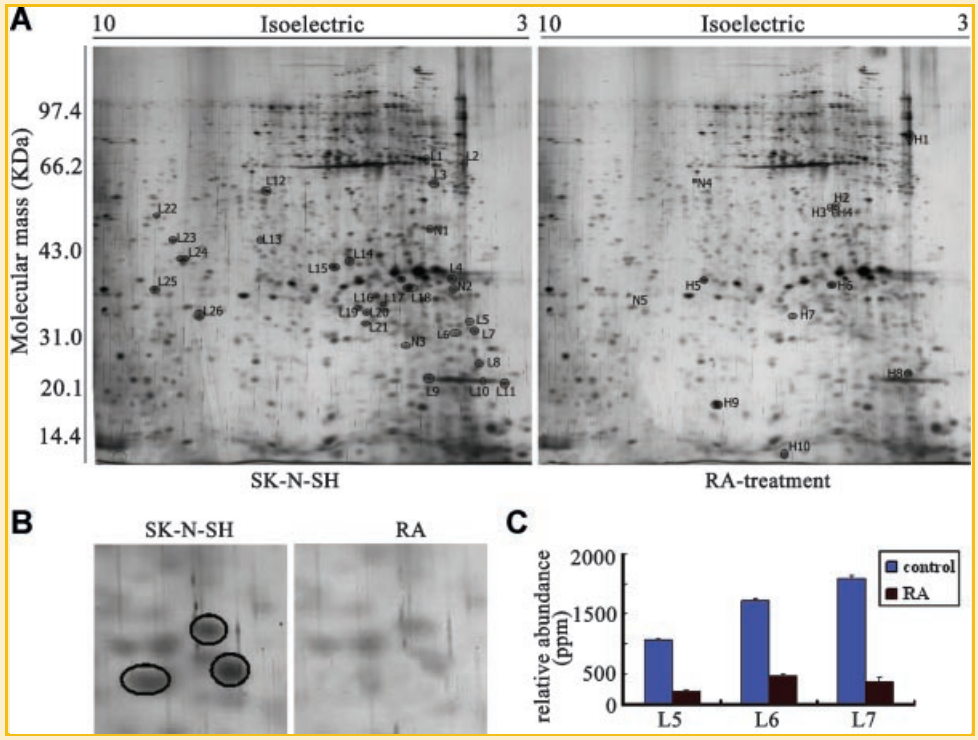

Fig. 1. 2D PAGE and altered NPM protein spots. A: 2D protein profiles from the nuclear matrix of SK-N-SH cells. Proteins were separated on the basis of pI (X-axis) and molecular mass (Y-axis) and visualized by silver staining. L5, L6, L7 indicate the spots of NPM (SK-N-SH: the nuclear matrix protein sample of SK-N-SH cells; RA treatment: the nuclear matrix protein sample of SK-N-SH cells treated with RA). B: Enlarged portions from 2DE gels. Circles indicate NPM protein spots. C: Optical density changes of identified NPM protein spots in the control and RA-treated SK-N-SH cells. Three repeated sets of silver-stained 2D gels were tested for each sample using PD Quest 8.0 software ($P < 0.02$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

enlarged detail of the NPM protein spot, and Figure 1C shows the relative expression level of NPM in the nuclear matrix pre- and post-treated with RA.

NPM DOWN-REGULATION AS SHOWN BY MALDI-TOF-MS IDENTIFICATION

PMFs of differentially expressed proteins were generated using MALDI-TOF-MS and then searched against the NCBInr and Swiss-Prot protein databases using the Mascot tool from Matrix Science. The protein spots (code no. L5/6/7) were identified as NPM, shown in Table I.

NPM DOWN-REGULATION AND ALTERED EXPRESSION OF C-MYC, C-FOS, P53, AND RB AS SHOWN BY WESTERN BLOTTING

The molecular weight of NPM was 38 kDa as shown in Figure 2. The NPM of the control group was highly expressed compared with the RA-treated group as determined by 2D PAGE.

C-myc, c-fos, p53, and Rb are differentiation-related genes and play pivotal roles in the regulation of cellular differentiation. Therefore, we analyze their expression levels and locations in SK-N-SH cells pre- and post-differentiation, as well as their colocalization with NPM. In the total proteins of SK-N-SH cells, the expression levels of c-myc and c-fos were down- regulated, while p53 and Rb were enhanced during the differentiation as shown in Figure 6.

NPM LOCALIZATION AND TRANSLOCATION IN THE NM-IF SYSTEM AS SHOWN BY FLUORESCENCE MICROSCOPY

The NPM in the NM-IF system was labeled with the fluorescent dye TRITC, showing as red fluorescence under a fluorescence microscope. The highly intensified NPM fluorescence was observed mainly in the residual nucleoli region of SK-N-SH cells, while the intensity of fluorescence was weak in the nuclear matrix and lamina regions (Fig. 3A). After treatment with RA, the distribution and expression of NPM were significantly altered. The intensity of fluorescence within the nucleus region decreased, especially in the

TABLE I. Proteins Identified Using the Mascot Database (www.matrixscience.com)

Spot no.	Protein name	Accession no.	Mol. mass (calc; Da)	pI (calc)	Score/coverage
L5	NPM	Q96EA5	32,726	4.64	73/25%
L6	NPM	Q96EA5	32,726	4.64	73/25%
L7	NPM	Q96EA5	32,726	4.64	73/25%

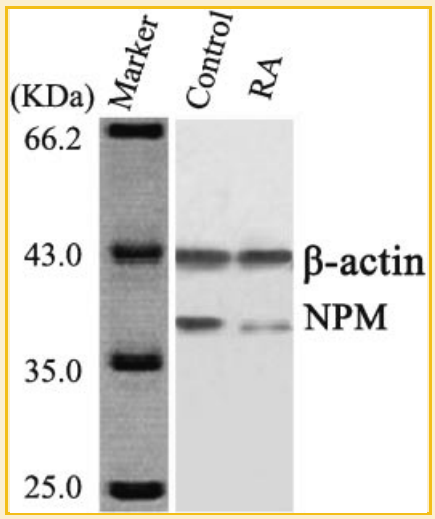

Fig. 2. Confirmation of NPM in the nuclear matrix of SK-N-SH cells by Western blotting. Western assay confirmed the existence and decreased expression of NPM protein reduced by RA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

nucleoli region, while that in the lamina and karyotheca regions increased. NPM also displayed a tendency to transfer from nucleolic regions to the nuclear matrix. Faint fluorescence could be observed even in intermediate filaments (Fig. 3B).

COLOCALIZATION OF NPM WITH C-MYC IN SK-N-SH CELLS

The result of laser scanning confocal microscopy showed that c-myc green fluorescence was assembled mainly in the nucleolus and its intensity varied significantly with respect to observations in the cytoplasm. Very little green fluorescence dispersed into the cytoplasm. There was no fluorescence detected in the center of the nucleolus, while the fluorescence was strong outside the nucleolus. Double immunofluorescence revealed that NPMs were colocalized with c-myc both inside and outside the nucleolus, but especially outside it (Fig. 4A–C). After treatment with RA, c-myc fluorescence intensity was weak, and dropped particularly around the nucleolus. Weak green fluorescence was uniformly distributed inside the cells. NPM colocalized with c-myc in the cellular cytoplasm, which indicates that the colocalization of NPM and c-myc transferred from the nucleolus to the cytoplasm (Fig. 4D–F).

COLOCALIZATION OF NPM WITH C-FOS IN SK-N-SH CELLS

The green fluorescence of c-fos was distributed through the whole cell. Compared with the cytoplasm, the fluorescence intensity in the nucleus was weak. Only a little colocalization of NPM and c-fos could be seen in the nuclear region (Fig. 4G–I). After treatment with RA, the intensity of green fluorescence weakened, and NPM evidently colocalized with c-fos in the cytoplasm and nucleolus, revealing that the colocalization of NPM and c-fos transferred from the nucleolus to the cytoplasm (Fig. 4J–L).

COLOCALIZATION OF NPM WITH P53 IN SK-N-SH CELLS

In human neuroblastoma SK-N-SH cells, the green fluorescence of p53 localized mainly in the nucleus and cytoplasmic region. Moreover, the fluorescence intensity of the nuclear region was much stronger than that of the cytoplasmic region. NPM colocalized with p53 in the nucleoplasm (Fig. 5A–C). After treatment with RA, the green fluorescence intensity of p53 in the cytoplasm increased, accumulating especially near the nucleus. The colocalization of NPM and p53 could be only observed during cytoplasmic processes. The results above demonstrate a tendency for the colocalization of NPM and p53 to transfer from nucleolic regions to the cytoplasm (Fig. 5D–F).

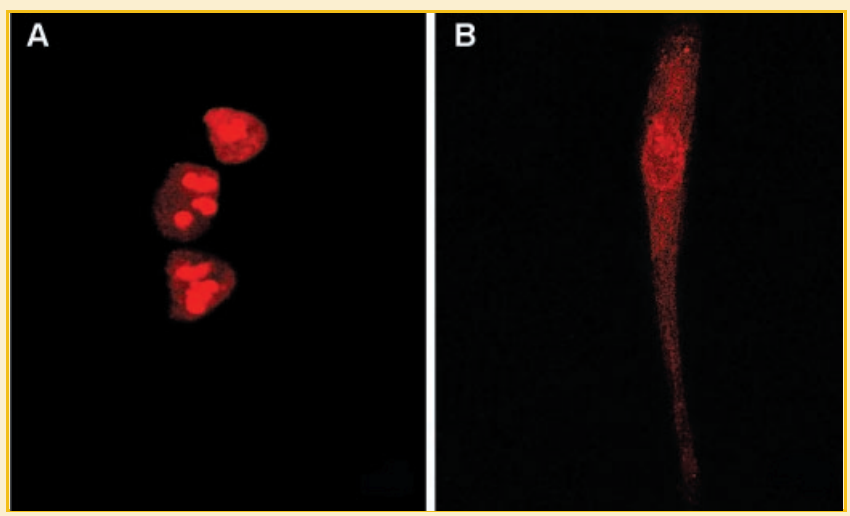

Fig. 3. Expression of NPM in the NM-IF system observed under a fluorescence microscope (the antibody of NPM labeled with fluorescent dye TRITC). Results of fluorescence microscopy showed that NPM in the NM-IF system of SK-N-SH cells had a positive reaction; red fluorescence was observed in the nuclear region, while fluorescence in the nucleolus region was relatively high (A). After treatment with RA, the intensity of red fluorescence in the nuclear region dropped and NPM fluorescence was observed in the lamina and cytoplasm (B). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

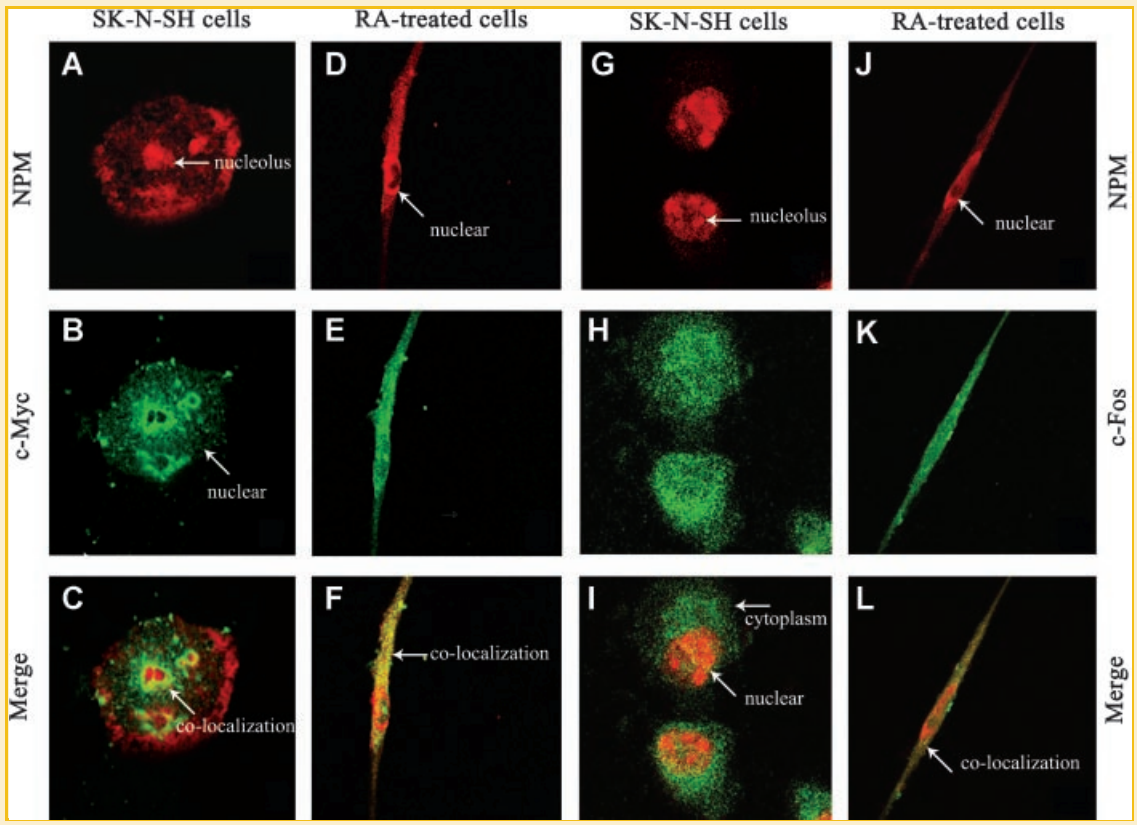

Fig. 4. Cellular colocalization of several oncogenes and NPM. A–F: Expression of NPM and c-myc in SK-N-SH cells. The overlay indicates the colocalization relationship between NPM and c-myc. G–L: Expression of NPM and c-fos in SK-N-SH cells. The overlay indicates the colocalization relationship between NPM and c-fos. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

COLOCALIZATION OF NPM WITH Rb IN SK-N-SH CELLS

The green fluorescence of Rb localized in the whole cell, accumulating mainly inside the nucleus, while the cytoplasmic intensity was weak. The colocalization of NPM and Rb could be observed in the nucleolus (Fig. 5G–I). After treatment with RA, the green fluorescence inside the whole cell was enhanced, accumulated in the nucleoplasm, and was weak in the cytoplasm. NPM mainly colocalized with Rb in the nucleoplasm and nearby nucleus. This indicates a tendency for colocalization of NPM and Rb to transfer from the nucleolus to the nucleoplasm (Fig. 5J–L).

DISCUSSION

LOCALIZATION AND EXPRESSION CHANGES OF NPM IN THE NUCLEAR MATRIX DURING THE DIFFERENTIATION OF SK-NK-SH CELLS

NPM is an important regulatory protein related to cell proliferation and growth. Its alteration of expression is closely correlated with cell carcinogenesis and its reverse. In this research, results from 2DE, MALDI-TOF analysis, and protein immunoblotting indicated that NPM was involved in the components of nuclear matrix proteins of human neuroblastoma SK-N-SH cells and its expression in the nuclear matrix of RA-induced cells was down-regulated, which was further proven by immunofluorescent microscopy. The existing research presents the multi-functions of NPM: NPM was over-expressed in a series of tumors and might be a proto-oncogene. Suppressing the expression of NPM through antisense oligonucleotides proved its important role in the anti-induced differentiation of tumor cells [Wang et al., 1998]. NPM had a tendency of down-regulated transcription in the RA-induced cell differentiation [Alsayed et al., 2001]. Moreover, NPM was thought to suppress the development of tumors. Its expression in normal cells was enhanced and activated the p53-dependent checkpoint; the knockout of NPM in mice would lead to the inactivation of p53 [Colombo et al., 2005]. NPM inhibited cell proliferation through interaction with the Arf protein. Our research data were consistent with the existing evidence that NPM was down-regulated during the differentiation of human tumor cells, as well as with our previous study [Li et al., 2008a,b], suggesting that NPM plays a regulatory role in the differentiation of human neuroblastoma SK-N-SH cells.

NPM is a nuclear matrix protein, and changes in its localization during cell differentiation might closely relate to its regulatory functions. In this research, data from 2DE and immunoblotting showed that NPM was one of the nuclear matrix components of human neuroblastoma SK-N-SH cells. The immunofluorescent microscopy results indicated that NPM accumulates mainly in the nuclear matrix, especially in the residue of the nucleolus. After

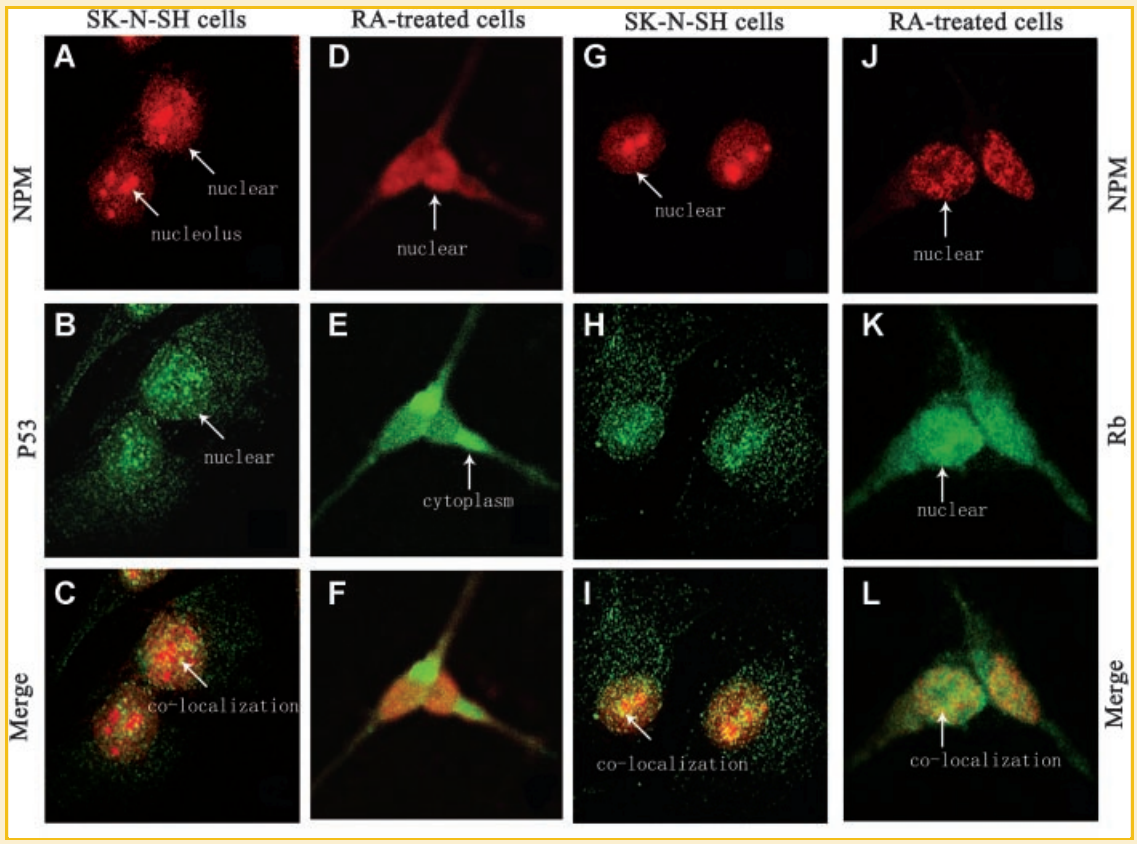

Fig. 5. Cellular colocalization of several tumor suppressor genes and NPM. A–F: Expression of NPM and p53 in SK-N-SH cells. The overlay indicates the colocalization relationship between NPM and p53. G–L: Expression of NPM and Rb in SK-N-SH cells. The overlay indicates the colocalization relationship between NPM and Rb. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

treatment with RA, the distribution pattern of NPM in the NM-IF system was obviously changed. The fluorescence signal in the nuclear area was weakened and uniformly scattered through the whole cell, suggesting a tendency of transport from the nucleolus to the cytoplasm. It is reported that NPM localized in the nucleolus and its localization was affected by the cell cycle and some cytotoxicity drugs. In the regeneration of liver cells, NPM translocated from the nucleolus to the karyoplasm, karyotheca, and cytoplasm [Subong et al., 1999]. However, its localization in the nuclear matrix is still unknown, and the altered localization in the nuclear matrix of tumor cells before and after induction has also yet to be discovered. Data presented in this article primarily showed the sub-localization of NPM. The changes of localization of NPM are closely correlated with the regulation of differentiation of SK-N-SH cells and might exert important functions in the RA-induced differentiation of human neuroblastoma SK-N-SH cells.

The functional research on NPM gene and its expression product has been a focus for years, but the transportation mechanism and its relationship with cell differentiation, as well as localization, are still unclear. In this article, we have proven that NPM is one of the components of nuclear matrix proteins, localizing in the nuclear matrix filament. The expression level and subcellular localization of NPM were altered after treatment with RA, indicating its pivotal role in the differentiation of human neuroblastoma SK-N-SH cells. Our research further provided experimental basis for research on carcinogenesis and its reverse in human neuroblastoma cells.

ALTERED COLOCALIZATION BETWEEN NPM AND ONCOGENES AND TUMOR SUPPRESSOR GENES

NPM is a member of the molecular chaperone family with nucleoplasmic [Dumbar et al., 1989] multi-functions. NPM affects cellular proliferation and differentiation through interaction with regulatory gene products. In this article, results from laser confocal microscopy revealed that NPM colocalized with c-fos, c-myc, p53, and Rb, indicating the potential interaction between them. Moreover, colocalization was altered after induced differentiation. The expression levels of c-myc and c-fos were down-regulated, while p53 and Rb were enhanced during the differentiation of SK-N-SH cells.

The overexpression of c-myc stimulates the cellular proliferation. C-fos interacts with c-jun and forms a heterodimer to regulate the growth, development, and differentiation of cells. It is reported that NPM directly interacts with c-myc, both of which are over-expressed in tumor and proliferating cells [Li et al., 2008c]. The expression of NPM mediated by c-myc [Yeh et al., 2006] was down-regulated after RA-induced differentiation [Yung, 2004]. However, the colocalization site of NPM with c-myc and the mechanism of

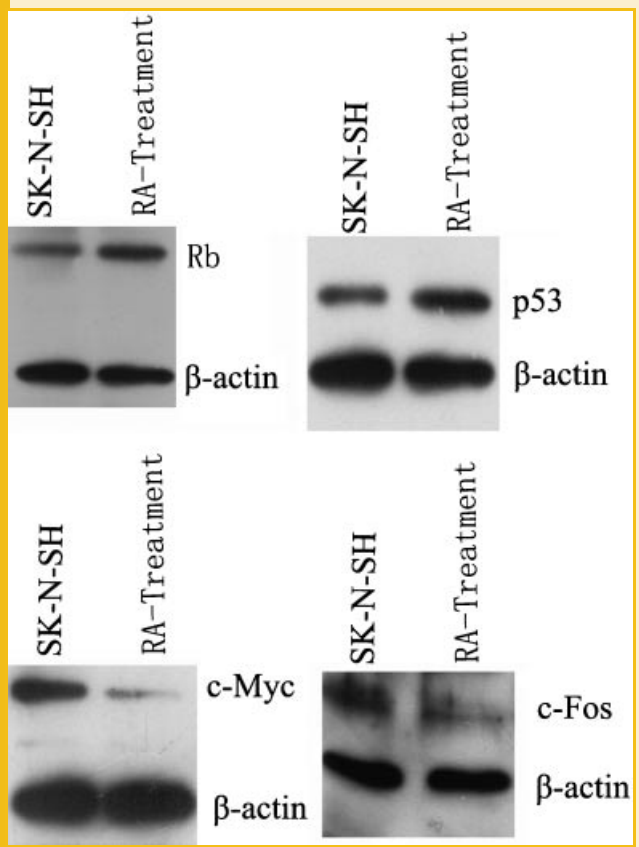

Fig. 6. Confirmation of altered expression of c-myc,c-fos,p53 and Rb in SK-N-SH cells by Western blotting. Western assay confirmed the expression levels of c-myc and c-fos were down- regulated, while p53 and Rb were enhanced during the differentiation of SK-N-SH cells.

down-regulated expression are still unknown. Our data revealed that NPM interacted with c-myc at the periphery of the nucleolus, while colocalization occurred in the cytoplasm after RA-induced differentiation. The changes in colocalization site reveal the alteration of interaction between NPM and c-myc, providing a supplement for elucidating the regulatory mechanism of NPM and c-myc in tumor development and differentiation. In this article, we first reported the altered colocalization of NPM with c-fos after induced differentiation. Our data showed the faint colocalization of NPM with c-fos in MG-63 cells, and their colocalized site was displaced from the nucleus to the cytoplasm, indicating a direct or an indirect interaction between them. We also found this change in other tumor cell lines [Li et al., 2008a]. Emanuela [Colombo et al., 2002] reported that NPM directly interacted with p53 and the overexpressed NPM altered the localization of p53 in WI38 cells, colocalizing with p53 in the nucleolus. Our data displayed that NPM colocalized with p53 in the nucleus of SK-N-SH cells and the colocalization occurred at the periphery of the nucleus. We inferred that the shuttle protein NPM binded with p53, induced its shuttle from the nucleus to the cytoplasm, and suppressed the accumulation of p53, as well as enhanced its stability. We still do not know the effects of altered colocalization in the regulation of human neuroblastoma SK-N-SH cell differentiation. Lin et al. [2006] reported that NPM in static bladder cancer cells interacted with hyperphosphorylated Rb at the early stages of serum stimulation. Our data further revealed the alteration of the colocalization site before and after differentiation. We provided evidence for further exploring the detailed regulatory mechanism of NPM through the Rb pathway. The alteration of NPM colocalizing with all the genes above in SK-N-SH cells indicates that NPM might affect the regulation of SK-N-SH cell carcinogenesis and its reverse through interacting with regulatory factors.

Altogether, our results prove the down-regulation of NPM expression in the NM-IF system in RA-induced differentiation. NPM colocalized with c-myc, c-fos, and Rb, as well as p53 in the nucleus. The colocalization of NPM with other genes was displaced from the nucleus to the periphery of the nucleus and cytoplasm, accompanied by the reverse of carcinogenesis phenotype. SK-N-SH cells underwent terminal differentiation. Our data revealed that NPM regulated the induced differentiation of human neuroblastoma SK-N-SH cells and interacted with the activation or inactivation of genes related to differentiation. We provided scientific supplement for the further study of the regulatory roles of NPM in differentiation, as well as carcinogenesis and its reverse in human neuroblastoma cells.

REFERENCES

Alsayed Y, Uddin S, Mahmud N, Lekmine F, Kalvakolanu DV, Minucci S, Bokoch G, Platanias LC. 2001. Activation of Rac1 and the p38 mitogen-activated protein kinase pathway in response to all-trans-retinoic acid. J Biol Chem 276:4012–4019.

Colombo E, Marine JC, Danovi D, Falini B, Pelicci PG. 2002. Nucleophosmin regulates the stability and transcriptional activity of p53. Nat Cell Biol 4:529–533.

Colombo E, Bonetti P, Lazzerini Denchi E, Martinelli P, Zamponi R, Marine JC, Helin K, Falini B, Pelicci PG. 2005. Nucleophosmin is required for DNA integrity and p19Arf protein stability. Mol Cell Biol 25:8874–8886.

Dumbar TS, Gentry GA, Olson MO. 1989. Interaction of nucleolar phosphoprotein B23 with nucleic acids. Biochemistry 28:9495–9501.

Frehlick LJ, Eirin-Lopez JM, Ausio J. 2007. New insights into the nucleophosmin/nucleoplasmin family of nuclear chaperones. Bioessays 29:49–59.

Li QF. 1999. Effect of retinoic acid on the changes of nuclear matrix in termediate filament system in gastric carcinoma cells. World J Gastroenterol 5:417–420.

Li QF, Shi SL, Liu QR, Tang J, Song J, Liang Y. 2008a. Anticancer effects of ginsenoside Rg1, cinnamic acid, and tanshinone IIA in osteosarcoma MG-63 cells: Nuclear matrix downregulation and cytoplasmic trafficking of nucleophosmin. Int J Biochem Cell Biol 40:1918–1929.

Li QF, Tang J, Liu QR, Shi SL, Chen XF, Song JY. 2008b. Expression and localization of nucleophosmin during HMBA-induced differentiation in human hepatocarcinoma SMMC-7721 cells. Chin J Biochem Mol Biol 2:8.

Li Z, Boone D, Hann SR. 2008c. Nucleophosmin interacts directly with c-Myc and controls c-Myc-induced hyperproliferation and transformation. Proc Natl Acad Sci USA 105:18794–9.

Lim MJ, Wang XW. 2006. Nucleophosmin and human cancer. Cancer Detect Prev 30:481–490.

Lin CY, Liang YC, Yung BY. 2006. Nucleophosmin/B23 regulates transcriptional activation of E2F1 via modulating the promoter binding of NF-kappaB, E2F1 and pRB. Cell Signal 18:2041–2048.

Michishita E, Kurahashi T, Suzuki T, Fukuda M, Fujii M, Hirano H, Ayusawa D. 2002. Changes in nuclear matrix proteins during the senescence-like

phenomenon induced by 5-chlorodeoxyuridine in HeLa cells. Exp Gerontol 37:885–890.

Naoe T, Suzuki T, Kiyoi H, Urano T. 2006. Nucleophosmin: A versatile molecule associated with hematological malignancies. Cancer Sci 97:963–969.

Subong EN, Shue MJ, Epstein JI, Briggman JV, Chan PK, Partin AW. 1999. Monoclonal antibody to prostate cancer nuclear matrix protein (PRO:4-216) recognizes nucleophosmin/B23. Prostate 39:298–304.

Wang DG, Liu WH, Johnston CF, Sloan JM, Buchanan KD. 1998. Bcl-2 and c-Myc, but not bax and p53, are expressed during human medullary thyroid tumorigenesis. Am J Pathol 152:1407–1413.

Ye K. 2005. Nucleophosmin/B23, a multifunctional protein that can regulate apoptosis. Cancer Biol Ther 4:918–923.

Yeh CW, Huang SS, Lee RP, Yung BY. 2006. Ras-dependent recruitment of c-Myc for transcriptional activation of nucleophosmin/B23 in highly malignant U1 bladder cancer cells. Mol Pharmacol 70:1443–1453.

Yun JP, Chew EC, Liew CT, Chan JY, Jin ML, Ding MX, Fai YH, Li HK, Liang XM, Wu QL. 2003. Nucleophosmin/B23 is a proliferate shuttle protein associated with nuclear matrix. J Cell Biochem 90:1140–1148.

Yung BY. 2004. c-Myc-mediated expression of nucleophosmin/B23 decreases during retinoic acid-induced differentiation of human leukemia HL-60 cells. FEBS Lett 578:211–216.