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The Localization of hnRNP A2/B1 in Nuclear Matrix and the Aberrant Expression During the RA-Induced Differentiation of Human Neuroblastoma SK-N-SH Cells

Ying Liang,[1] Song-Lin Shi,[2] Qi-Fu Li,[1*] Lan-Ying Chen,[3] Guang-Jun Jing,[1] Guo-Wei Tan,[4] San-Ying Wang,[1] and Fu-Yun Wu[1]

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

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

[3]*Henan University of Urban Construction, Pingdingshan 467044, China*

[4]*The Affiliated First Hospital of Medical College, Xiamen University, Xiamen 361005, China*

ABSTRACT

Heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 is involved in the synthesis of RNA. Its expression is up-regulated in many tumor cell lines. In this study, we investigated the distribution of hnRNP A2/B1 in the nuclear matrix, including its co-localization with expression products of related genes. Results from 2-DE PAGE and MS showed that hnRNP A2/B1 is involved with components of nuclear matrix proteins of SK-N-SH cells, and that its expression level is down-regulated after retinoic acid (RA) treatment. Protein immunoblotting results further confirm the existence of hnRNP A2/B1 in the nuclear matrix, as well as its down-regulation after RA treatment. Immunofluorescence microscopy observation showed that hnRNP A2/B1 localized in nuclear matrix of SK-N-SH cells and its distribution regions were altered after RA treatment. Laser scanning confocal microscopy observation showed that hnRNP A2/B1 co-localized with c-Myc, c-Fos, P53, and Rb in SK-N-SH cells. The co-localized region was altered as a result of RA treatment. Our data proved that hnRNP A2/B1 is a nuclear matrix protein and can be up-regulated in human neuroblastoma. The expression and distribution of hnRNP A2/B1 can affect the differentiation of SK-N-SH cells, as well as its co-localization with related oncogenes and tumor suppressor genes. J. Cell. Biochem. 112: 1722–1729, 2011.

KEY WORDS: hnRNP A2/B1; NUCLEAR MATRIX; HUMAN NEUROBLASTOMA; DIFFERENTIATION

The heterogeneous nuclear ribonucleoproteins family of eukaryotic cells consists of about 20 members; it can form RNP complexes by combining with precursor RNA. The hnRNP A2/B1 is a key member of the hnRNP family, and is derived from a single-copy gene localized in human chromosome 7. P15, protein B1 (37 kDa), and A2 (34 kDa) were produced for the alternative splicing of the second exon. Moreover, protein B1 and A2 could form a complex at a rational proportion [Kozu et al., 1995]. It has also been reported that hnRNP A2/B1 could regulate cellular migration through alternatively splicing pre-mRNA [Moran-Jones et al., 2009]. hnRNP A2 could bind to single DNA, participate in the maintenance of the telomere structure, and regulate DNA duplication, transcription, and recombination [Moran-Jones et al., 2005]. hnRNP B1 could also affect the genome stability by suppressing the activity of DNA-dependent protein kinase (DNA-PK), as well as regulate cell cycle and apoptosis [Han et al., 2008]. Moreover, hnRNP B1 may also be involved in the ubiquitin regulatory pathway and closely relate to the differentiation of some cells [Maggipinto et al., 2004; Ma et al., 2009]. As a tumor marker, hnRNP A2/B1 is overexpressed in a variety of tumors, an example of which is lung cancer [Satoh et al., 2000; Zhou et al., 2001; Sato et al., 2008]. hnRNP A2/B1 also interacts with the Wig-1 protein induced by p53, suggesting that hnRNP A2/B1 might be closely related with the tumor inhibitory roles of p53 [Prahl et al., 2008].

At present, the roles of hnRNP A2/B1 in the carcinogenesis and reversal of tumor cells are still unclear. The mechanism undertaken

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

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

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by hnRNP A2/B1 in cellular proliferation and differentiation still lacks systematic research. Our previous study has found an aberrant expression of hnRNP A2/B1 in nuclear matrix-intermediate filament system during the induced-differentiation of human osteosarcoma MG-63 cells and human neuroblastoma SK-N-SH cells, suggesting that hnRNP A2/B1 is closely related with the proliferation and differentiation of human neuroblastoma SK-N-SH cells [Zhao et al., 2008; Liang et al., 2009]. Therefore in this study, retinoic acid (RA), a classical inducer [Liang et al., 2009; Tonge and Andrews, 2010], was used to induce human neruoblastoma SK-N-SH cells into differentiation. We investigated the distribution and expression of hnRNP A2/B1 in the nuclear matrix, and primarily explored the relationship of hnRNP A2/B1 with related oncogenes and tumor suppressor genes. This study aimed to provide more evidences for the roles of hnRNP A2/B1 in tumor cell differentiation.

MATERIALS AND METHODS

CELL CULTURE

Human neuroblastoma SK-N-SH cells from China Center for Type Culture Collection were cultured 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, SK-N-SH cells were maintained in a RPMI-1640 with 1 μM RA (Sigma) for 7 days to induce differentiation. SK-N-SH cells cultured in RPMI-1640 medium were taken as a control.

CELLULAR NUCLEAR MATRIX–INTERMEDIATE FILAMENT (NM–IF) SYSTEM SAMPLES

Cells on cover slips were selectively extracted as described by Liang et al. [2009].

PREPARATION OF NUCLEAR MATRIX PROTEIN SAMPLES (NMPs)

The selective extraction method was optimized as described by Li [1999]. SK-N-SH cells were washed twice with 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 washed with cold PBS to remove soluble cytoplasmic proteins and then re-centrifuged and suspended in the digestion buffer CSK50 (identical to CSK100 buffer, except with 50 mM NaCl instead of KCl) containing 400 U/ml DNase I for 30 min at room temperature. Cold ammonium sulfate was added at a final concentration of 0.25 M to precipitate proteins.

After centrifugation at 1,000*g* for 5 min, the pellets were washed with CSK50 buffer and 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 groups were determined by the Bradford assay, and the proteins were stored at −80°C.

2-DE PAGE AND GEL ANALYSIS

2-DE PAGE was performed using standard methods [Lin et al., 2008]. After silver-staining, the gels were scanned with an image scanning equipment (UMAX Power Look III) and analyzed using PD Quest 8.0 software (Bio-Rad). The intensities of protein spots were normalized to the total intensity of the entire gel. Protein spots with at least twofold changes in intensity were defined as differentially expressed nuclear matrix proteins.

MALDI-TOF-MS ANALYSIS AND hnRNP A2/B1 IDENTIFICATION

The differentially expressed protein spots were cut from the gels. After silver removal, reduction with DL-dithiothreitol, alkylation with iodacetamide, and inner-gel digestion with trypsin, the protein samples were subjected to a MALDI-TOF mass spectrometer (ReFlex^{TM} III, Bruke). Mass finger print data were obtained and treated using the flexAnalysis software to remove pollution peaks and to correct instrumental error by autolysis peaks of trypsin. Corrected data were used to search in NCBInr and Swiss-Prot protein databases by employing the Mascot tool from Matrix Science.

WESTERN BLOTTING

Protein lysates were electrophoretically separated from 12% polyacrylamide gels and transferred onto PVDF membranes. The membranes were incubated in non-fat milk for 1.5 h at room temperature to block any unspecific binding and incubated overnight at 4°C with mouse hnRNP A2/B1 monoclonal antibody (1:1,000, Santa Cruz Biotechnology) in TBST. The membranes were then washed three times with TBST for 30 min, incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (1:10,000; Santa Cruz Biotechnology) for 45 min, and washed three times with TBST. Immunoreactive bands were identified using an enhanced chemiluminescence detection system (Pierce). Two percent BSA was used as negative control instead of the primary antibodies. β-Actin was taken as an internal control.

SAMPLE PREPARATION FOR IMMUNOFLUORESCENT MICROSCOPE

The NM–IF system of RA-treated SK-N-SH cells was selectively extracted. After washing with PBS, the NM–IF system 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 hnRNP A2/B1 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 mounted with a drop of anti-fluorescence quencher. Three percent BSA was used as negative control instead of the primary antibody.

LASER SCANNING CONFOCAL MICROSCOPY OBSERVATION OF hnRNP A2/B1

SK-N-SH cells and RA-treated cells were seeded on the cover slip and continuously cultured for another 2–3 days. After washing twice with PBS for 10 min, the cells were immersed in 0.5% Triton X-100 for 5–10 min. After fixing with 4% paraformaldehyde for 10 min, the cells were blocked with 3% BSA for 1 h. The cover slips

were incubated with different primary antibody mixtures (hnRNP A2/B1/c-myc, hnRNP A2/B1/c-fos, hnRNP A2/B1/p53, and hnRNP A2/B1/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 as the negative group instead of the primary antibody.

RESULTS

RESULTS OF 2-DE PAGE AND IDENTIFICATION OF MALDI-TOF-MS

Nuclear matrix proteins extracted from SK-N-SH cells before and after RA-treatment were subjected to 2-DE PAGE, and the procedure was independently repeated three times (Fig. 1A). Scanned gels were quantified using the PDQuest 8.0 version (Bio-Rad). The differentially expressed protein spot was analyzed by MALDI-TOF-MS and the mass fingerprints of the acquired peptides were matched against peptide data (www.matrixscience.com). The spot coded L25 was identified as hnRNP A2/B1 (Fig. 1B; Table I) and the expression of hnRNP A2/B1 was observed to have reduced in the nuclear matrix of the treated cell (Fig. 1C).

THE CONFIRMATION OF PROTEIN IMMUNOBLOTTING

Nuclear matrix proteins were separated by SDS–PAGE and transferred onto PVDF membrane for Western blot analysis. The antibody dilution without primary antibody was taken as a negative control. No protein band was detected in the negative control. After incubation with primary antibody, the protein band was only detected around the target protein molecular weight, suggesting the good specificity of the primary antibody. hnRNP A2/B1 consisted of two different proteins. The molecular weight of hnRNP B1 was 37 kDa at the N termination, of which were 12 more amino acids than hnRNP A2. Meanwhile, the molecular weight of hnRNP A2 was 34 kDa. However, Western blot assay results mainly showed the

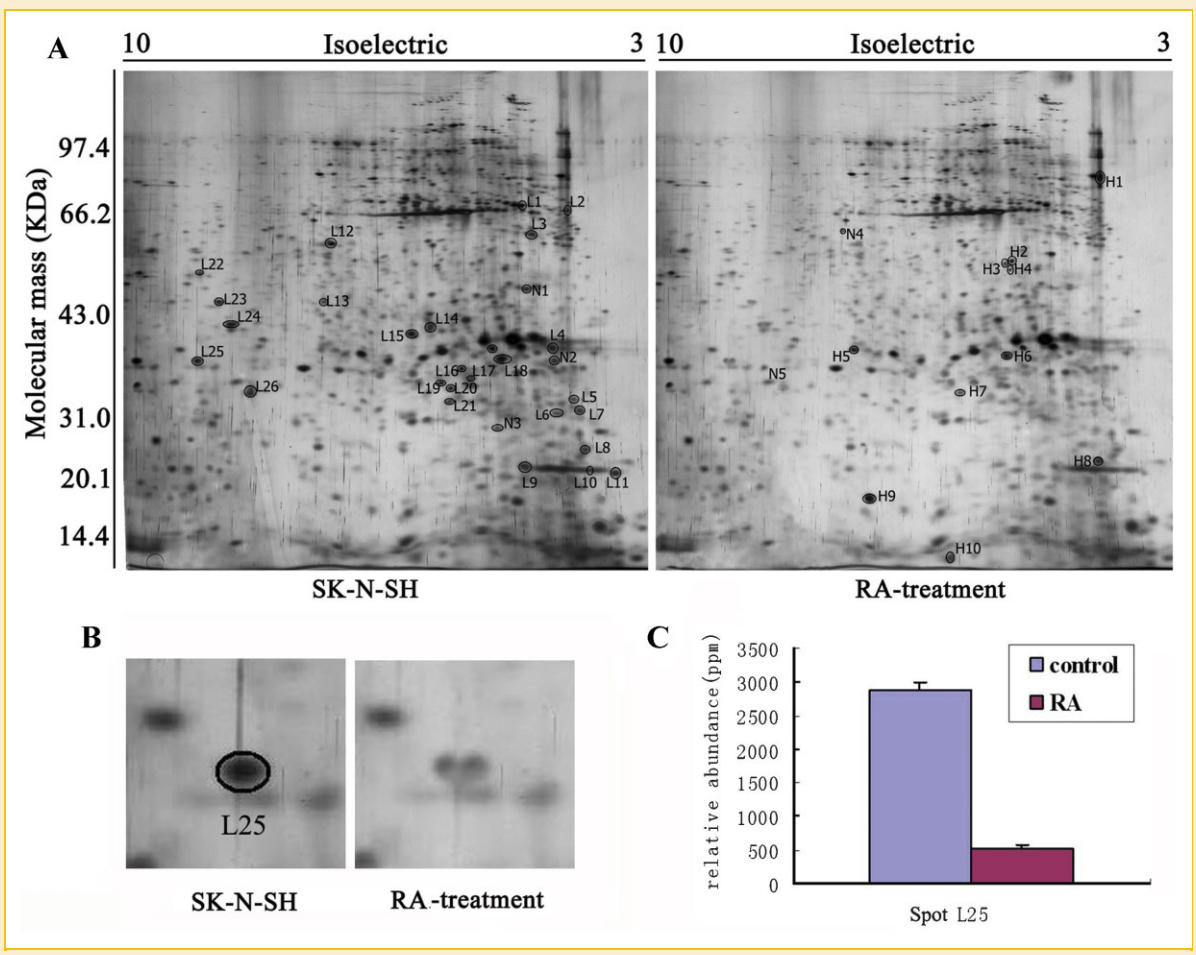

Fig. 1. 2D PAGE and altered hnRNP A2/B1 protein spot. A: 2D protein profiles from the nuclear matrix of SK-N-SH cells. Proteins were separated on the basis of pI (*X*-axis) and molecular mass (*Y*-axis), and visualized by silver staining. L25 indicates the spot of hnRNP A2/B1 (SK-N-SH: the nuclear matrix 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 hnRNP A2/B1 protein spot. C: Optical density changes of identified hnRNP A2/B1 protein spot 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 ($P < 0.02$).

TABLE I. The hnRNP A2/B1 Protein Spot Identified by Using MASCOT Database

Spot no.	Protein name	Accession no.	Mol. mass calc (Da)	pI (calc)	Score/coverage
L25	hnRNP A2/B1	P22626	36,600	8.67	101/43%

protein band of hnRNP B1, although its expression level was down-regulated after induced differentiation (Fig. 2).

THE LOCALIZATION AND EXPRESSION OF hnRNP A2/B1 IN NUCLEAR MATRIX-INTERMEDIATE FILAMENT SYSTEM OF SK-N-SH CELLS

The hnRNP A2/B1 in SK-N-SH cells before and after RA treatment was labeled with fluorescent dye TRITC, which showed red fluorescence under fluorescence microscope. Results proved that the intensity of red fluorescence in the nuclear matrix of human neuroblastoma SK-N-SH cells was strong and was distributed throughout the whole nuclear matrix-intermediate filament system. The red fluorescence was stronger in the nucleus but weak in the cytoplasm (Fig. 3A). After RA treatment, the density and distribution of red fluorescence representing hnRNP A2/B1 were both altered in the nuclear matrix-intermediate filament system. The density of red fluorescence was weakened throughout the entire cell, especially in the nucleus region and the nucleolus, where it disappeared completely, suggesting that the distribution of hnRNP A2/B1 was transported from the nucleolus to the region of karyoplasms (Fig. 3B).

THE CO-LOCALIZATION OF hnRNP A2/B1 WITH c-Myc, c-Fos, P53, AND Rb IN THE CELLS

SK-N-SH cells were doubly immunostained with primary antibodies of hnRNP A2/B1 and c-Myc, c-Fos, P53, and Rb. The antibody against hnRNP A2/B1 was labeled with red fluorescence TRITC while the other antibodies were labeled with green fluorescence FITC. Laser confocal scanning microscope was employed to observe the alteration in co-localization of hnRNP A2/B1 with related gene products. The co-localized region was shown to be yellow or orange-yellow.

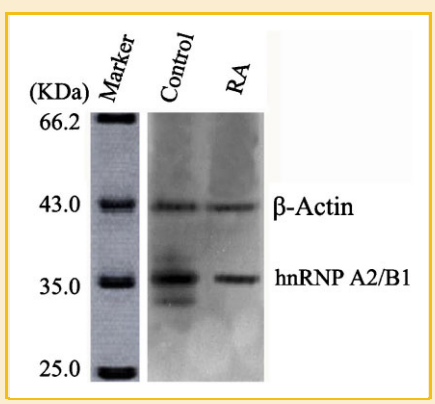

Fig. 2. Confirmation of hnRNP A2/B1 in the nuclear matrix of SK-N-SH cells by Western blotting. Western assay confirmed the existence and decreased expression of hnRNP A2/B1 reduced by RA.

In the control cells, the red fluorescence representing hnRNP A2/B1 was distributed throughout the SK-N-SH cells. The fluorescence density in the nucleus area was relatively strong and aggregated in the nucleolus. The fluorescence in the cytoplasm, on the other hand, was much weaker than in the nucleus. After RA treatment, the red fluorescence mainly distributed in the nucleus but not in the nucleolus. Scattered fluorescence was weakened in the cytoplasm, even disappeared.

In the control cells, the green fluorescence representing c-Myc strongly distributed in the nucleus, although the density was not uniform. In the cytoplasm, only little scattered green fluorescence was detected. The fluorescence in the nuclear matrix was uniform. The density of fluorescence in the peripheral of the nucleolus was strong. No fluorescence was detected in the central area of the nucleolus. After the red and green fluorescence were merged together, results showed that hnRNP A2/B1 co-localized with c-Myc in the nuclear matrix region, especially in the nucleolus region (Fig. 4A–C). After RA treatment, the green fluorescence of c-Myc was entirely weakened and uniformly distributed in the whole cells. The merged fluorescence showed that the co-localization of hnRNP A2/B1 with c-Myc in the nucleus is not obvious, suggesting that the co-localization between hnRNP A2/B1 and c-Myc disappeared from the nucleolus (Fig. 4D–F).

In the control cells, the green fluorescence representing c-Fos was strongly distributed in the nuclear matrix region, especially in the nuclear membrane area. In the cytoplasm, only little scattered green fluorescence was observed. After the red and green fluorescence were merged together, results showed that hnRNP A2/B1 co-localized with c-Fos in the nuclear matrix region, especially in the nucleolus region (Fig. 4G–I). After RA treatment, the green fluorescence of c-Fos was entirely weakened and uniformly distributed in the whole cells. The merged yellow fluorescence suggested that the co-localization of hnRNP A2/B1 with c-Fos in the nucleus was weakened, indicating that the co-localization of hnRNP A2/B1 with c-fos disappeared from the nucleolus (Fig. 4J–L).

In the control cells, the green fluorescence representing P53 was entirely weak and scattered throughout the cell. The density of fluorescence at the peripheral of the nucleus was relatively weak, while fluorescence in the nucleolus region was stronger. After the red and green fluorescence were merged together, the result showed hnRNP A2/B1 co-localized with p53 in the nuclear matrix region, especially in the nucleolus region (Fig. 4M–O). After RA treatment, the green fluorescence was enhanced and mainly distributed in the peripheral of the nucleus and in the cytoplasm. The density of green fluorescence in the nucleus was weak. The merged fluorescence suggested that the co-localization of hnRNP A2/B1 with P53 was not

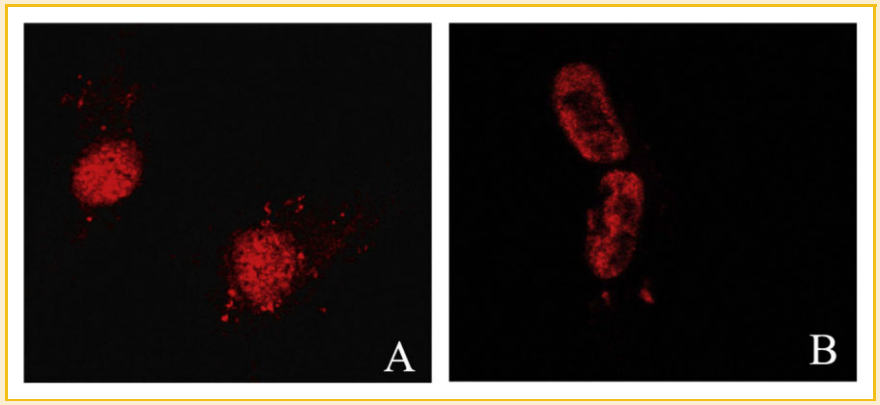

Fig. 3. Expression of hnRNP A2/B1 in the NM–IF system observed under fluorescence microscope (the antibody of hnRNP A2/B1 is labeled with TRITC). A: Results of fluorescence microscopy show that hnRNP A2/B1 in the NM–IF system of SK-N-SH cells gave a positive reaction; red fluorescence distributed in the whole nuclear matrix-intermediate filament system. The red fluorescence was stronger in the nucleus but weak in the cytoplasm. B: After RA treatment, the intensity of red fluorescence decreased and disappeared in the nucleolus. The distribution of hnRNP A2/B1 was transferred from the nucleolus to the region of karyoplasms.

obvious; the orange-yellow fluorescence was only seen in the nucleus, indicating that the co-localized region of hnRNP A2/B1 with P53 gradually disappeared from the nucleolus (Fig. 4P–R).

In the control cells, the green fluorescence representing Rb was entirely weak and scattered throughout the cell. The density of fluorescence at the peripheral of the nucleus was relatively weak, while the fluorescence in the nucleolus region was stronger. The merged yellow fluorescence showed that hnRNP A2/B1 co-localized with Rb in the nuclear, especially in the nucleolus region (Fig. 4S–U). After RA treatment, the green fluorescence of Rb was enhanced and mainly distributed in the nuclear. The merged fluorescence suggested that the co-localization of hnRNP A2/B1 with Rb was in the nuclear but disappeared from the nucleolus (Fig. 4V–X).

DISCUSSION

hnRNP A2/B1 is a nuclear matrix protein localizing in nuclear matrix fibers. Tumor cellular differentiation is followed by the aberrant expression and altered-localization of hnRNP A2/B1. Our study proved the existence and down-regulated expression of hnRNP A2/B1 in the components of nuclear matrix of RA-treated cells. HnRNP A2/B1 was mainly expressed in the nuclear matrix but not in the nucleolus, and its expression was dramatically down-regulated after RA treatment. A clear transfer pattern from nucleolus to nuclear matrix was observed. Our data primarily illustrated the subcellular distribution of hnRNP A2/B1. Changes of protein expression level determined by the fluorescence intensity were consistent with protein immunoblotting results. This suggests that the expression alteration and subcellular distribution closely related to the differentiation process of SK-N-SH cells. As an important differentially expressed nuclear matrix protein, hnRNP A2/B1 might play pivotal roles in the regulation of human neuroblastoma SK-N-SH cellular differentiation.

In this article, Western blotting results proved that the protein expression level of hnRNP B1 was higher than that of hnRNP A2 in human neuroblastoma SK-N-SH cells. The down-regulation of hnRNP B1 was particularly marked, while hnRNP A2 was even undetectable after RA treatment. The relationship between hnRNP A2/B1 and cancer development has already been reported in an early research on lung cancer. Sueoka et al. [1999] produced the antibodies for hnRNP A2 and hnRNP B1 and found that the enhancement of hnRNP A2/B1 in lung cancer cells was mainly caused by the improvement of hnRNP B1, which was later taken as indicator for lung cancer [Pino et al., 2003].

The aberrant expression of hnRNP A2/B1 was also found in a variety of tumor cell lines including liver caner, pancreatic cancer, breast cancer, and gastric cancer [Yan-Sanders et al., 2002; Garayoa et al., 2003; Snead et al., 2003; Turck et al., 2004]. Deep investigation concerning hnRNP A2/B1 showed that hnRNP A2/B1 participates in the regulation of cellular growth and it was over-expressed at the pre-cancerous stage, suggesting that the over-expression of hnRNP A2/B1 closely related to the malignant proliferation of tumor cells [Satoh et al., 2000; He et al., 2005]. In this article, the aberrant expression of hnRNP A2/B1 in SK-N-SH cells before and after the reversal of malignant phenotype was consistent with the reports regarding other tumor cell lines, and we further proved the close relationship between hnRNP A2/B1 and the malignant proliferation of tumor cells and suggested that the expression of hnRNP A2/B1 related to the differentiation of SK-N-SH cells. We also found that the aberrant expression of hnRNP A2/B1 was caused by the changes concerning hnRNP B1, which was consistent with the research on human lung cancer and other tumor cells, suggesting the mechanism undertaken by hnRNP A2/B1 in the reversal and carcinogenesis of human neuroblastoma SK-N-SH cells was similar with that in lung cancer cells.

In addition, hnRNP A2/B1 localized in the nucleus, and its over-expression and localization in the cytoplasm was the biomarker of lung cancer. The methylation on nuclear localization signal sequence of hnRNP A2 affected its cellular localization [Nichols et al., 2000]. However, the relation between hnRNP A2/B1 and the nuclear matrix has not yet been reported. Our recent study found that the localization and quantity of hnRNP A2/B1 in the nuclear matrix could be significantly altered during the differentiation of

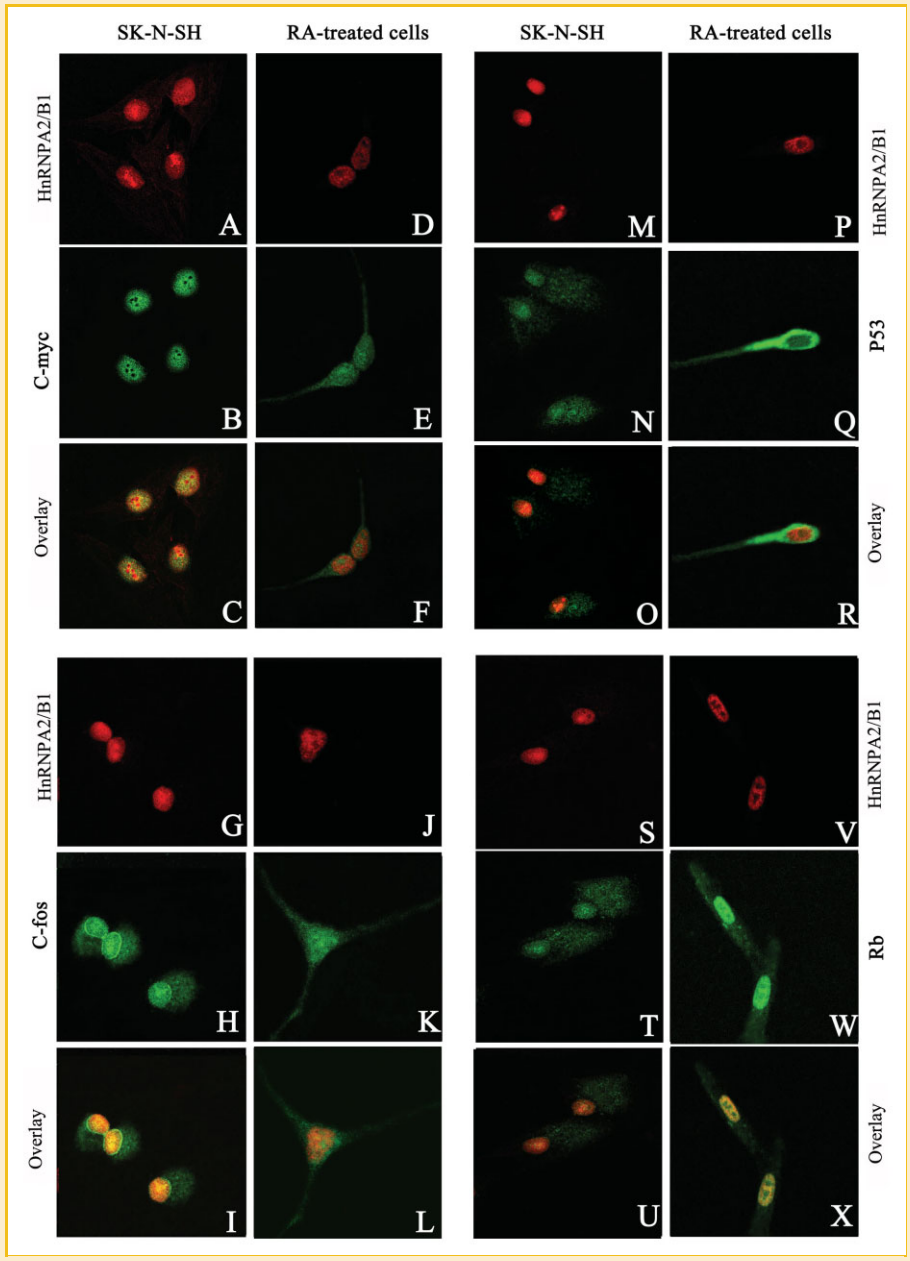

Fig. 4. Cellular co-localization of hnRNP A2/B1 with several oncogenes and tumor suppressor genes. A–F: Expression of hnRNP A2/B1 and c-myc in SK-N-SH cells. The yellow fluorescence of the overlay indicates the co-localization relationship between hnRNP A2/B1 and c-myc. G–L: Expression of hnRNP A2/B1 and c-fos in SK-N-SH cells. The yellow fluorescence of the overlay indicates the co-localization relationship between hnRNP A2/B1 and c-fos. M–I: Expression of hnRNP A2/B1 and p53 in SK-N-SH cells. The yellow fluorescence of the overlay indicates the co-localization relationship between hnRNP A2/B1 and p53. S–X: Expression of hnRNP A2/B1 and Rb in SK-N-SH cells. The yellow fluorescence of the overlay indicates the co-localization relationship between hnRNP A2/B1 and Rb.

human osteosarcoma MG-63 cells and human neuroblastoma SK-N-SH cells induced with HMBA, RA, and the effective ingredients of Chinese medicine (RCT) (data to be published). We supposed that hnRNP A2/B1 was transferred to the nuclear matrix from the nucleolus in some undefined mechanism during the induced differentiation of human neuroblastoma SK-N-SH cells. Moreover, the translocation of hnRNP A2/B1 affected its regulation on cell proliferation and differentiation. The over-expression of hnRNP A2/B1 might be closely related with vigorous proliferation of SK-N-SH cells, as well as the aberrant expression of oncogenes and tumor suppressor genes.

We initially found that hnRNP A2/B1 was part of the components of nuclear matrix proteins and its expression level and subcellular localization were altered after RA-treatment. Our results provided further evidences for the research on the mechanism of hnRNP A2/B1 in SK-N-SH nuclear matrix and its relation with the oncogenes and tumor suppressor genes. Deep investigation on the mechanism of hnRNP A2/B1 in the induced differentiation of tumor

cells will help to reveal its detailed functions in cellular carcinogenesis.

hnRNP A2/B1 played pivotal roles in cellular senescence, proliferation, and differentiation [Satoh et al., 2000; Zhu et al., 2002; Turck et al., 2004; He et al., 2005]. The aberrant expression of hnRNP A2/B1 in various tumor cells and its relation with telomeres suggested that it played important regulatory roles in the carcinogenesis of tumors [Satoh et al., 2000; Moran-Jones et al., 2005]. The interaction between hnRNP A2/B1 and some functional proteins was necessary for the function of hnRNP A2/B1 [D'Ambrogio et al., 2009].

Our data showed that hnRNP A2/B1 co-localized with c-Myc, c-Fos, P53, and Rb. After the RA-induced treatment, the co-localized region was altered. The expression levels of c-Myc and c-Fos were down-regulated, while P53 was up-regulated, suggesting hnRNP A2/B1 participated in the regulation of SK-N-SH cellular proliferation through interacting with the expression products of oncogenes and tumor suppressor genes.

Proto-oncogenes c-myc and c-fos are closely related with cellular malignance. The aberrant expression of c-Myc and c-Fos might lead to cellular malignant transformation. On the other hand, over-expression of c-Myc and c-Fos was found in many kinds of tumor cells. The aberrant activation of c-myc would prompt cells into S phase from G0/G1 phase. When c-Fos was accidentally activated, the thymidine kinase-promoting factor (TKPA), which was the target gene of c-fos and the positive regulator of hnRNP A2/B1, was over-expressed, and further promoted the expression of hnRNP A2/B1 [Kozu et al., 1995]. At present, the relationship of hnRNP A2/B1 with c-Myc and c-Fos was still undefined. Our research for the first time found the intracellular co-localization and transformation of hnRNP A2/B1 with c-Myc and c-Fos in the nuclear matrix of SK-N-SH cells upon RA induction, suggesting hnRNP A2/B1 might interact with c-Myc and c-Fos and bind together to form complexes. We further inferred that hnRNP A2/B1 might participate in tumor differentiation through synergy with c-Myc and c-Fos, although the detailed mechanism was still unclear. The mutation of p53 and its over-expression were common in tumor cells. Rb regulated the activity of E2F by forming complexes with E2F and affected cellular proliferation. hnRNP A2/B1 was involved in the regulation of P53, the tumor suppressor, by interacting with p53-induced protein Wig-1 [Prahl et al., 2008]. Our research for the first time presented the co-localization of hnRNP A2/B1 with P53 and Rb, suggesting hnRNP A2/B1 regulated the differentiation of neuroblastoma SK-N-SH cells through a close interaction with p53 and Rb.

In conclusion, our data presented in this research show that RA destabilizes nuclear matrix and affects localization of hnRNP A2/B1 from nucleolus to nuclear matrix, and the malignant phenotype of human neuroblastoma was reversed and cellular proliferation was inhibited by RA. Meanwhile, hnRNP A2/B1 co-localizes with the c-Myc, c-Fos, P53, and Rb in human neuroblastoma cells, and both the subcellular location and expression level of hnRNP A2/B1 are changed during RA-induced differentiation. These results suggest that hnRNP A2/B1 might participate in the regulatory process of tumor cell proliferation and differentiation through alternating its distribution and interacting with the expression products of oncogenes and tumor suppressor genes.

Our study primarily analyzed and revealed the aberrant location and expression of hnRNP A2/B1 in the RA-induced differentiation of SK-N-SH cells, as well as the interaction of hnRNP A2/B1 with oncogenes and tumor suppressor genes. In our previous study, the expression level of hnRNP A2/B1 was down-regulated in the induced-differentiation of MG-63 cells and BGC-823 cells, suggesting it should be a key protein in the regulation of tumor cell differentiation. Further investigation regarding the interaction of hnRNP A2/B1 with the expression products of genes is of great importance for the research on cellular proliferation and differentiation.

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