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Basic Research Paper

Expression and localization of hnRNP A2/B1 during differentiation of human osteosarcoma MG-63 cells induced by HMBA

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Key words: HnRNP A2/B1, nuclear matrix, osteosarcoma, MG-63 cell, cell differentiation

Background and Objective: Differentially expressed nuclear matrix proteins have great effects on canceration and regulation of cell differentiation. This study was to explore the existence and distribution of ribonucleoprotein hnRNP A2/B1 in the nuclear matrix and its co-localization with Actin and Prohibitin in human osteosarcoma MG-63 cells before and after hexamethylene bisacetamide (HMBA) treatment. Methods: The nuclear matrix of MG-63 cells before and after treatment of HMBA was selectively extracted. The expression and localization of hnRNP A2/B1 in the nuclear matrix were detected by 2-D PAGE, MALDI-TOF-MS, Western blot and immunofluorescent staining. Co-localization of hnRNP A2/B1 with Actin and Prohibitin was observed under a laser scanning confocal microscope (LSCM). Results: hnRNP A2/B1 was detected in the component of nuclear matrix proteins of MG-63 cells by Western blot and immunogold staining and its expression was decreased after treatment of HMBA. hnRNP A2/B1 was located in the nuclear matrix, and its expression was weakened after HMBA treatment. hnRNP A2/B1 was co-localized with Actin or Prohibitin in MG-63 cells, while the co-localization was weakened during differentiation of MG-63 cells. Conclusions: The distribution and expression of hnRNP A2/B1 and its co-localization with Actin and Prohibitin play important roles during the differentiation of MG-63 cells.

Ribonucleoprotein HnRNPA2/B1 is one of the pre-mRNA-binding proteins. As a core member of hnRNP family, hnRNPA2/B1 is not only associated with mRNA translocation and post-transcriptional modification, but also related to DNA replication, transcription and recombination through binding to single-stranded DNA (ssDNA).[1-4] Moreover, changes in the expression of hnRNPA2/B1 affect differentiation and cancerization of cells. In addition, HnRNPA2/B1 is also a potential marker for early diagnose of tumors.[5-8] Previously we have claimed that hnRNP A2/B1, whose expression was altered during hexamethylene bisacetamide (HMBA)-induced differentiation, is a component of the nuclear matrix of human osteosarcoma MG-63 cells and human gastric adenocarcinoma MGC80-3 cells.[9,10] We propose that hnRNP A2/B1, which has a close relationship with proliferation and differentiation of cancer cells, is a nuclear matrix protein.

HMBA is a compound with small polar molecules. Recently it is identified as an effective differentiation inducer of many kinds of human tumors, including leukemia, osteosarcoma, hepatocarcinoma and gastric carcinoma. Our previous research has shown that HMBA has remarkable influence on the differentiation of MG-63 cells.[11, 12] Therefore, in this study, we investigated the localization and expression of hnRNPA2/B1 in the nuclear matrix of MG-63 cells, and analyzed the correlation of hnRNPA2/B1 to Prohibitin, a potential tumor suppressor protein, and actin, a component of nuclear matrix proteins. Thus, we may have a better understanding of the role of hnRNPA2/B1 in cell proliferation and differentiation, and provide scientific evidence for further exploration of cell cancerization and its reversion of the cell.

Materials and Methods

Materials. MG-63 cells were purchased from China Center for Type Culture Collection, Wuhan University. Rabbit anti-human hnRNPA2/B1 was bought from Santa Cruz Co.; mouse anti-human Actin, goat anti-human IgG-TRITC, goat anti-rabbit IgG-TRITC, goat anti-mouse IgG-Cy3 were all provided by Boster Co.; mouse anti-human Prohibitin was bought from NeoMarkers Co, USA. RPMI-1640 medium was the product of Gibco Co.; newborn calf serum was from Hangzhou Sijiqing Biological Engineering Materials Co. Ltd. HMBA was purchased from Sigma Co., USA.

Cell culture and induction of differentiation. MG-63 cells were cultured at 37°C in RPMI-1640 medium (pH 7.2) supplemented with 10% newborn calf serum, 100 U/mL penicillin, 100 U/mL streptomycin and 50 μg/mL kanamycin. Cells were passaged at log phase of growth. After 24 h, cells of the treatment group were changed with culture media containing 5 mmol/L HMBA. Culture media was

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changed every 48–72 h and cells were harvested after several passages.

Selective extraction of cells and sample preparation for light microscopy. Cells were selectively extracted as described in Li et al.[13] The nuclear matrix- intermediate filament (NM-IF) samples on the cover slip after selective extraction were prefixed in 2% glutaraldehyde at 4°C for 30 min, and rinsed with phosphate-buffered saline (pH 7.4). Cells were stained with 0.2% coomassie brilliant blue for 20 min, washed with distilled water, dried in air, clarified in xylene, enveloped in a resin, and observed under a Olympus BH-2 microscope.

Purification of nuclear matrix proteins. Nuclear matrix proteins of MG-63 cells were purified using the routine method with minor modifications.[14]. MG-63 cells were washed with PBS and suspended in cytoskeleton buffer (CSK100) (10 mmol/L PIPES pH 6.8, 300 mmol/L sucrose, 100 mmol/L NaCl, 4 mmol/L $CaCl_2$, 1.0 mmol/L PMSF, 0.5% Triton X-100) at 0°C for 10 min, and subjected to centrifugation for 5 min at 400 g. The deposition was washed twice with CSK50 (10 mmol/L PIPES pH 6.8, 300 mmol/L sucrose, 50 mmol/L NaCl, 4 mmol/L $CaCl_2$, 1.0 mmol/L PMSF, 0.5% Triton X-100) and digested in CSK50 containing 300 U/mL DNase I for 30 min at 25°C. 1 mol/L ammonium sulfate (NH_4) was added drop-wise to yield a final concentration of 0.25 mol/L. After incubation for 15 min, nuclear matrix proteins were pelletted by centrifugation at 1000 r/min for 5 min. After rinse with CSK50, proteins were stored at -80°C.

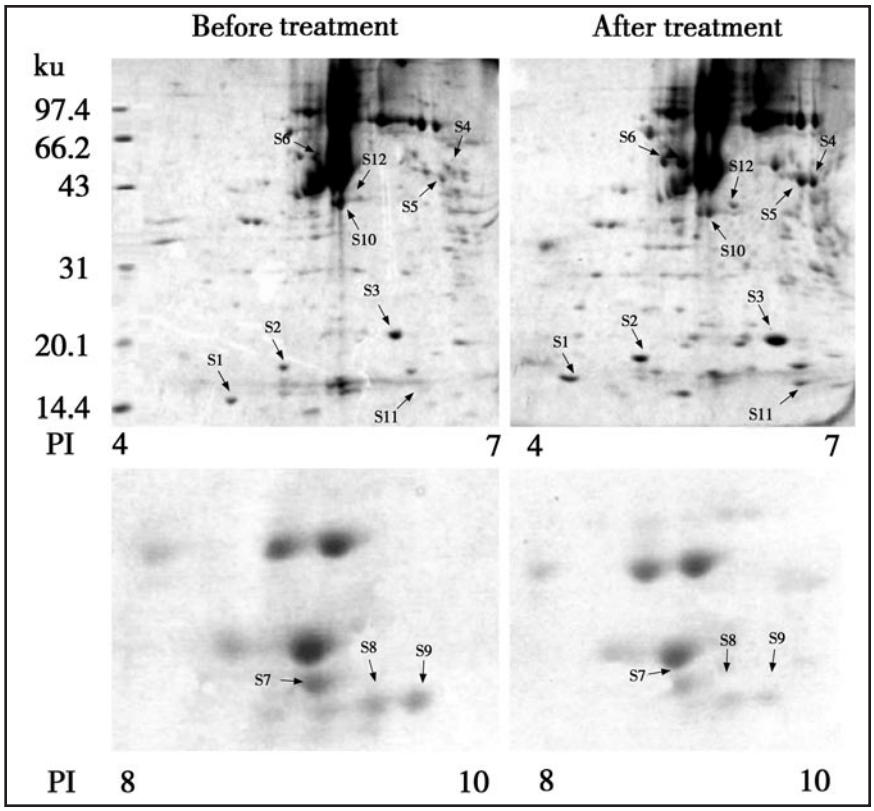

Figure 1. Expression of nuclear matrix proteins in MG-63 cells before and after HMBA treatment on 2-D PAGE gels. Top, pI 4–7, silver staining; Bottom, enlarged maps of changed expression of hnRNP A2/B1, pI 8–10, silver staining.

Two-dimensional gel electrophoresis (2-DE) and image analysis. The 2-DE was proceeded according to the conventional method. Isoelectric focusing (IEF) was performed in ReadyStrip IPG strips (pH 3-10, Bio-Rad Co.), while a 4% stacking gel and a 12.5% resolving gel were used for SDS-PAGE. Gels were stained with silver nitrate protocol compatible with mass spectrometry MS. Three sets of repeated 2-D maps of nuclear matrix proteins were analyzed using PDQuest (Bio Rad) software. After spot detection, spot matching, background subtraction and standardization of the intensity of each spot, the part of the gel map, which represented the hnRNP A2/B1 spot, was enlarged.

MALDI-TOF-MS analysis of nuclear matrix proteins. Silver-stained spots were cut out from the gels. After silver removal, reduction with DL-Dithiothreitol, alkylation with iodacetamide, and in-gel digestion using trypsin, solution containing extracted tryptic peptides was obtained. Samples were dissolved for MALDI-TOF-MS analysis. Mass measurements were performed on a Brucker ReFlexTM III MALDI-TOF mass spectrometer. The PMF data were analyzed using flexanalysis software to calibrate and remove polluted peaks. Peptide mass fingerprints obtained by the MALDITOF- MS were used to search NCBI or Swiss-Prot database using Mascot software from Matrix Science (www.matrixscience.com).

Western blot. Nuclear matrix proteins were separated by SDS-PAGE electrophoresis and then transferred onto PVDF membranes. Non-specific reactivity was blocked by incubation of the membrane with 5% bovine serum albumin (BSA) buffer at room temperature for 1.5 h. The membrane was then incubated with anti-hnRNP A2/B1 (1:1000) antibody at room temperature for 2 h. After being washed, the secondary antibody (combined with horseradish peroxidase) was added. Reactive protein was detected by enhanced chemiluminescence (ECL) detection system (Pierce). For a negative control, the primary antibody was replaced by 5% BSA buffer. Anti β-actin polyclonal antibody was probed as an internal loading control.

Sample preparation for fluorescence microscopy. NM-IF samples on the cover slip were prefixed in 4% paraformalclehyde at 4°C for 10 min, rinsed with TPBS containing 0.5% Triton X-100 twice, 5 min each time, blocked by 5% BSA at room temperature for 1 h, incubated with anti-hnRNP A2/B1 (1:1000) antibody at room temperature for 30 min, 4°C overnight, followed by rinse with TPBS for three times. Slips were incubated with the secondary antibody labeled with fluorescence dye FITC, washed with water and air-dried. Subsequently, the slip was enveloped in 90% glycerol/PBS and then observed under fluorescence microscopy. Processes after incubation with the secondary antibody were performed in dark. For a negative control, the primary antibody was replaced by 5% BSA buffer.

Sample preparation for LSCM. Cells on the cover slip were rinsed with PBS, submerged in TBS containing 0.5% Triton X-100 for 30 min. After rinse with PBS, cells were fixed in 4% paraformalclehyde for 10 min, blocked by 5% BSA at room temperature for 1 h, and then incubated with dual primary antibodies at room temperature for 30 min and then 4°C overnight. The dual primary

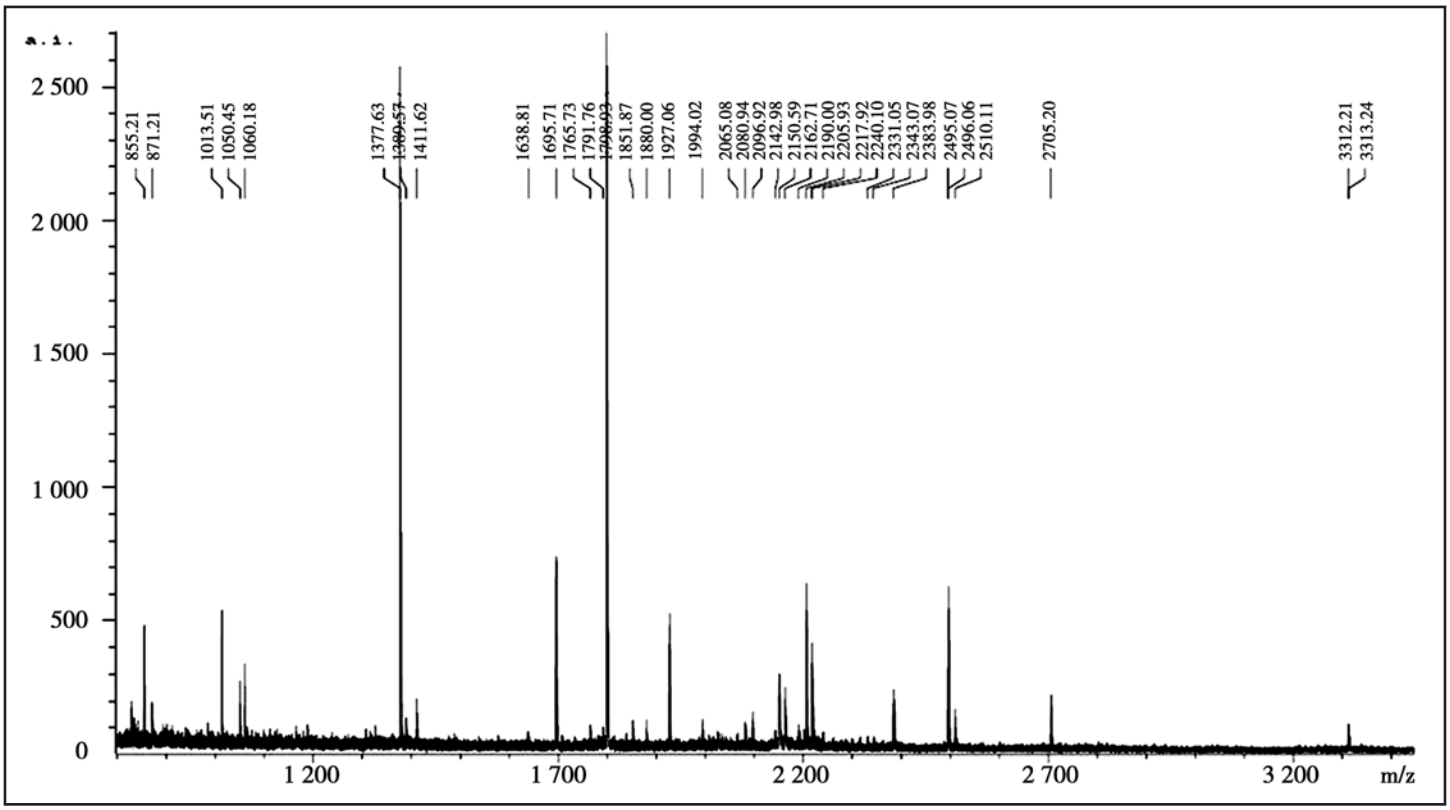

Figure 2. Peptide mass fingerprinting (PMF) of S7 protein

antibody sets were comprised of anti-hnRNP A2/B1 (1:1000)/actin (1:200), anti-hnRNP A2/B1/prohibitin (1:200). After being washed with TTBS, cells were incubated with different sets of secondary antibodies labeled with FITC, TRITC or Cy3, respectively at room temperature for 30 min and then at 4°C overnight. The slip was washed with TPBS, enveloped in 90% glycerol/PBS after air drying and then observed under a laser scanning confocal microscope (LSCM) (TCS-SP2 MP).

Results

Differential expression levels of hnRNP A2/B1 before and after HMBA treatment in the nuclear matrix of MG-63 cells. The 2-DE of nuclear matrix extracts of MG-63 cells was performed for more than three times. Figure 1A shows 2-DE gel images of acidic terminal proteins, while Figure 1B shows the 2-DE gel images of alkaline terminal proteins of the nuclear matrix extracted from MG-63 cells before and after HMBA treatment. Nuclear matrix proteins were mainly distributed at the isoelectric point of 4–7, with the molecular weight of 10–100 ku, most of which were between 40 ku and 100 ku. After treatment with HMBA, the number of proteins became much fewer at the alkaline terminal than at the acidic terminal, and only a few protein spots were observed scattered in the gel (spot S7 was identified as hnRNP A2/B1 by MALDI-TOF-MS). The intensity of spot S7 was dramatically reduced by two-fold in HMBA-treated MG-63 cells compared to the control.

Results of MALDI-TOF-MS analysis. After MALDI-TOF-MS analysis and MASCOT database search, the protein spot S7 obtained from 2-DE gels was identified as hnRNP A2/B1 (Fig. 2). Relevant information was extracted from the website (www.matrixscience.com): NCBI entry, gi|4504447; MW, 36041Da; PI, 8.67; sequence coverage, 37%; matching peptides, 8.

Effects of HMBA on the protein expression of hnRNP A2/B1. The immunoband of hnRNP A2/B1 (37 ku) of control MG-63 cells was obviously fuscous, which became light and thin in HMBA-treated cells. The expression level of hnRNP A2/B1 was significantly decreased by several folds after treatment of HMBA. The expression level of β-actin (42 ku), the endogenous control, remained unchanged (Fig. 3).

Observation of the localization and expression of hnRNP A2/B1 in the NM-IF system of MG-63 cells. Light microscopy observation revealed that the intermediate filaments in untreated MG-63 cells were sparse and irregularly arranged. Compared with control, in HMBA-treated MG-63 cells, the whole framework became more stretched; the NM-IF system showed characteristics of uniform distribution of untransformed cells; the intermediate filaments, stained uniformly, spread from the region around nucleus to the cellular edge and formed a well-distributed and regular network throughout the cytoplasm; filaments of the nuclear matrix were abundantly and evenly distributed (Fig. 4A and B). The green immunofluorescence of hnRNP A2/B1 was mainly, unevenly distributed in the nuclear region of MG-63 cells. Strong fluorescence was observed in parts of the nuclei of control MG-63 cells (Fig. 5A). After incubation with HMBA, the expression of hnRNP A2/B1 in the NM-IF system was decreased dramatically. The holistic intensity of hnRNP A2/B1 immunofluorescence dropped in the nuclear matrix region in particular. The fluorescence intensity in the nuclei of HMBA-treated cells was much fainter than that in untreated MG-63 cells (Fig. 5B).

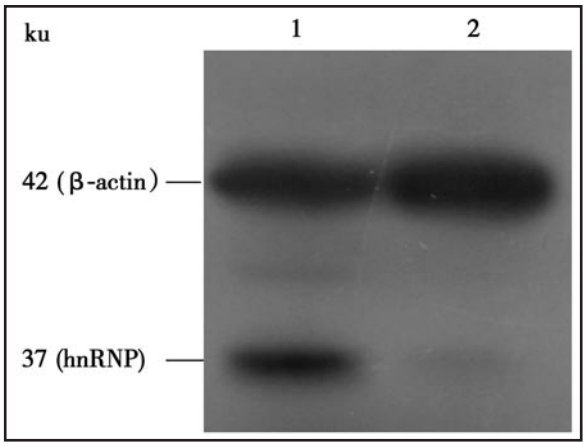

Figure 3. Expression of hnRNP A2/B1 in the nuclear matrix of MG-63 cells. Lane 1, untreated MG-63 cells; lane 2, HMBA-treated MG-63 cells.

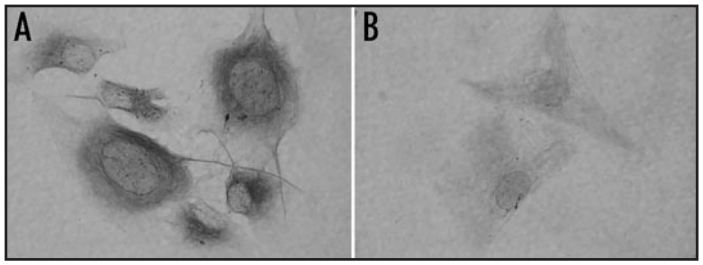

Figure 4. Nuclear matrix-intermediate filaments (NM-IF) system in MG-63 cells observed under light microscopY (Coomassie brilliant blue ×400). (A) The distribution of nuclear matrix-intermediate filaments in MG-63 cells is irregular. (B) The distribution of nuclear matrix-intermediate filaments in MG-63 cells is relatively regular after HMBA treatment.

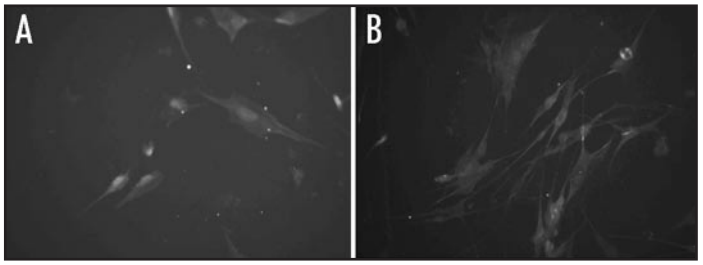

Figure 5. Expression of hnRNP A2/B1 in the NM-IF system of MG-63 cells observed under fluorescence microscopy (×200). (A) The expression of hnRNP A2/B1 in the NM-IF system of MG-63 cells is relatively strong. (B) The expression of hnRNP A2/B1 in the NM-IF system of MG-63 cells is weaker after HMBA treatment.

Co-localization of hnRNP A2/B1 with Actin or Prohibitin. Co-localizations of hnRNP A2/B1 with Prohibitin or Atin were observed by a LSCM. hnRNP A2/B1 was labeled with FITC (green), Actin was labeled with TRITC (red), and Prohibitin was labeled with Cy3(red). Co-localized fluorescence was yellow or orange when two different fluorescence overlapped.

Co-localization of hnRNP A2/B1 with actin in MG-63 cells. In control MG-63 cells, the green fluorescence of hnRNP A2/B1 was mainly distributed in the nuclei, and its intensity was comparatively strong. The red fluorescence of Actin was distributed in the cytoplasm (primarily) and the nuclear region. The yellow overlapped fluorescence indicated that hnRNP A2/B1 and Actin were mainly co-localized in the area near the karyotheca (Fig. 6A–C). In HMBA-treated MG-63 cells, the green fluorescence of hnRNP A2/B1 became weaker; little, scatted fluorescence was observed occasionally in the cell. The red fluorescence was widely and fascicularly located in the cell. The overlapped fluorescence indicated a decrease of co-localization of hnRNP A2/B1 with Actin after treatment of HMBA (Fig. 6D–F).

Co-localization of hnRNP A2/B1 with Prohibitin in MG-63 cells. In control MG-63 cells, green fluorescence of hnRNP A2/B1 was mainly distributed in the nuclear region and the fluorescence intensity was uneven, exhibiting a point-shape distribution. Red fluorescence of Prohibitin was distributed primarily in the nuclear region as well. The yellow overlapped fluorescence indicated that hnRNP A2/B1 and Prohibitin were strongly co-localized in the nuclear region (Fig. 6G–I). In HMBA-treated MG-63 cells, the green fluorescence of hnRNP A2/B1 was decreased. The intensity of red fluorescence was weakened in the nuclei but strengthened in the cytoplasm. The overlapped fluorescence indicated that co-localization of hnRNP A2/B1 and Prohibitin was attenuated in the nuclear region (Fig. 6J–L).

Discussion

hnRNP A2/B1 is a regulator, which affects the cell growth, differentiation and protein synthesis. There are different distributive characteristics of hnRNP A2/B1 in tumor cells compared to normal cells. The detailed localization of hnRNP A2/B1 in cells has not yet been clarified. In this study, using selective extraction of cells and 2-DE analysis, we discovered that hnRNP A2/B1 existed in the nuclear matrix extract of MG-63 cells. Moreover, hnRNP A2/B1 was observed to be mainly localized in the nuclear matrix of MG-63 cells with uneven distribution. The expression of hnRNP A2/B1 was relatively high in some local areas. Our previous studies have shown that hnRNP A2/B1 not only is related to splicing of pre-mRNA and nucleocytoplasmic transportation,[15] but also participates in the regulation of cell differentiation and tumorigenesis.[16,17] Studies on localization of hnRNP A2/B1 has not been reported so far. The only relevant study reveals that hnRNP U exists and adheres to RNA in the nuclear matrix.[18] We have previously found that hnRNP A2/B1 is expressed in the nuclear matrix of both MG-63 and MGc80-3 cells, implying that hnRNP A2/B1 might be a nuclear matrix protein.[9,10] We conclude from this study that hnRNP A2/B1 is a nuclear matrix protein, which localizes in the nuclear matrix of MG-63 cells.

The expression level of hnRNP A2/B1 associates closely to cell cancerization. We found that the expression level of hnRNP A2/B1 in the nuclear matrix was much higher in MG-63 cells than in HMBA-treated MG-63 cells, which was further proven by western blot. hnRNP A2/B1 was confirmed to be located in the NM-IF system of MG-63 cells, whose expression and distribution were altered during HMBA-induced differentiation. The configuration of the NM-IF system in tumor cells is irregular and not well distributed; compositions of the nuclear matrix alter at different stages of differentiation, which differs from normal cells.[19,20] Recent studies have claimed that hnRNP A2/B1 is of great relevance in cancerization. For example, in many tumors, such as lung carcinoma, hepatocarcinoma, pancreatic carcinoma, breast carcinoma and gastric carcinoma and

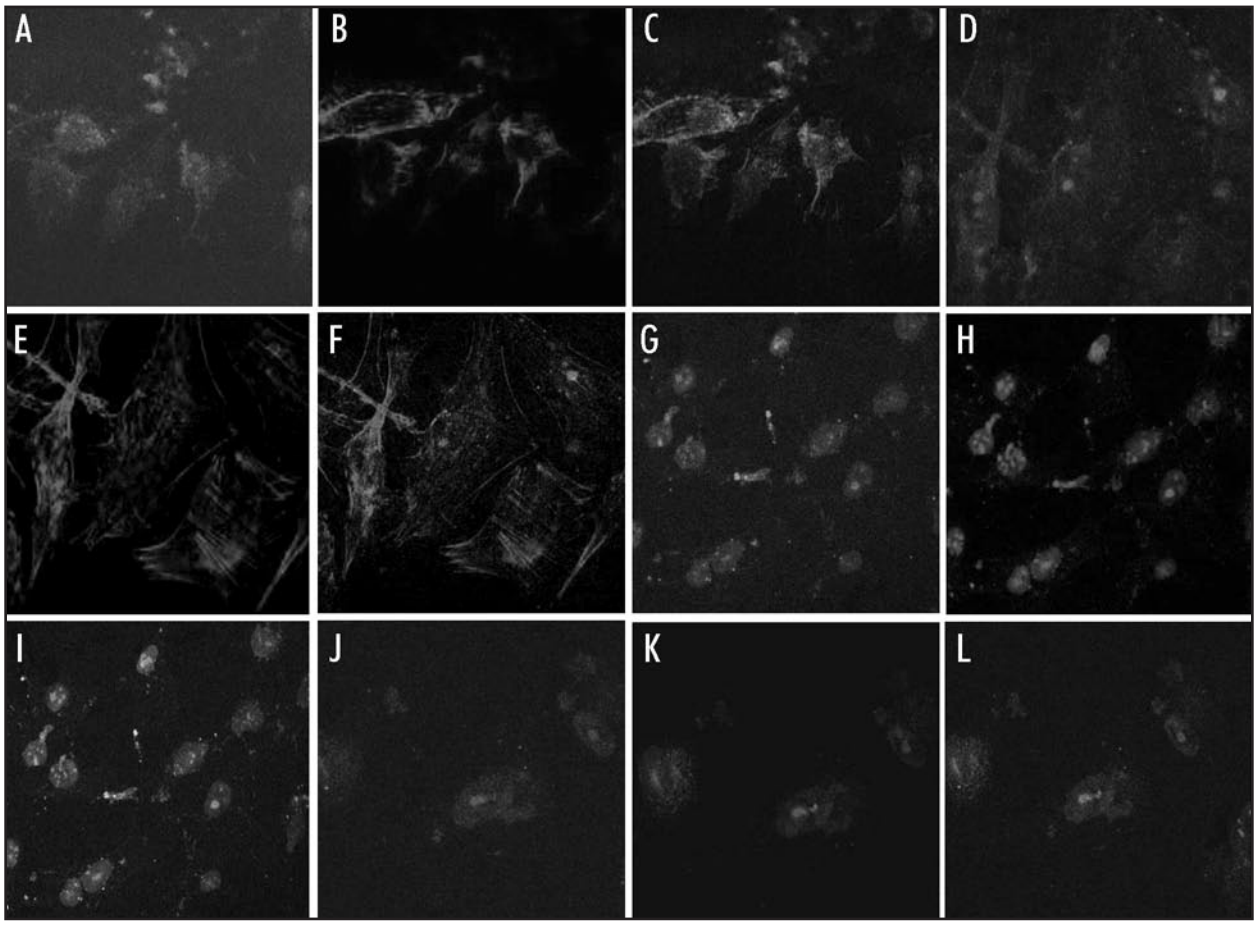

Figure 6. Co-localization of hnRNP A2/B1 with Actin and Prohibitin in MG-63 cells before and after treatment of HMBA observed under laser scanning confocal microscopy (×400). (A) hnRNP A2/B1 is strongly expressed in nuclei of MG-63 cells. (B) Actin is strongly expressed in cytoplasm of MG-63 cells. (C) hnRNP A2/B1 and Actin are co-localized in the karyotheca of MG-63 cells. (D) hnRNP A2/B1 is weakly expressed in nuclei of MG-63 cells after HMBA treatment. (E) Actin is mostly expressed in the cytoplasm and the karyoplasm of MG-63 cells after HMBA treatment. (F) Co-localization of hnRNP A2/B1 and Actin in MG-63 cells is weakened after HMBA treatment. (G) hnRNP A2/B1 is strongly expressed in nuclei of MG-63 cells. (H) Prohibitin is strongly expressed in nuclei and weakly expressed in the cytoplasm of MG-63 cells. (I) hnRNP A2/B1 and Prohibitin are strongly co-localized in nuclei of MG-63 cells. (J) hnRNP A2/B1 is weakly expressed in nuclei of MG-63 cells after HMBA treatment. (K) The expression of Prohibitin is weak in nuclei and relatively strong in the cytoplasm of MG-63 cells after HMBA treatment. (L) Co-localization of hnRNP A2/B1 and Actin in nuclei of MG-63 cells is weakened after HMBA treatment.

so on, hnRNP A2/B1 is usually abnormally expressed. An increase of hnRNP A2/B1 is confirmed to be through the upregulation of hnRNP B1.[8,16] Differential expression of hnRNP A2/B1 was detected during differentiation of MG-63 cells, which was in accordance with other studies. Thus, it is suggested that hnRNP A2/B1 has participated in the regulation of HMBA-induced differentiation of MG-63 cells.

Alterations of expression and distribution of hnRNP A2/B1 is significant to the proliferation and differentiation of MG-63 cells. hnRNP A2/B1 could interfere and regulate the process of differentiation by interacting with other regulators in tumor cells. Co-localization of hnRNP A2/B1 with Actin in the region near the karyotheca in control MG-63 cells was exhibited in this study, the density of which was weakened after HMBA treatment. Meanwhile, hnRNP A2/B1 and Prohibitin were co-localized at specific regions, especially in the nuclei of MG-63 cells. The density of fluorescence also became weakened in HMBA-treated cells. It is reported that Actin not only acts as an important structural protein of cells, but also contributes to the stability of structural filaments. Moreover, the expression level of Actin in the nuclear is essential and important to maintain the structure and function of nucleus.[21,22] Actin plays a crucial role in connecting the nuclear skeleton and the intermediate filament system, as well as in maintaining the morphology of cell skeleton.[23] Our LSCM results displayed co-localized fluorescence of hnRNP A2/B1 with Actin in the region close to the nuclear membrane of MG-63 cells, implying a possible direct interaction of the two in this region; whereas in HMBA-treated cells, such co-localization become weakened, which might be explained by downregulation of both proteins, or reconstruction of the NM-IF system during the induction of differentiation. As a potential tumor suppressor gene, Prohibitin influences various aspects of cell activity, including maintenance of the mitochondrial function, regulation of cell cycle and transduction signaling. The subcellular distribution, modification and expression level of Prohibitin also have important impact on the proliferation and differentiation of tumor cells. Prohibitin has become a target protein in studying cancerization and tumor diagnosis. In recent years, it has been found that Prohibitin could block the gene expression and regulation,

reduce the proliferation of cells through interacting with related oncogenes or tumor suppressor genes, and recruiting/suppressing transcriptional factors.[24,25] Our results imply that the association between hnRNP A2/B1 and Prohibitin might get involved in the regulation of proliferation and differentiation of tumor cells, and thus worthy of further investigation. It is proposed that hnRNP A2/B1 is not only an important regulator of proliferation and differentiation, but is also involved in tumor reversion of MG-63 cells through its subcellular distribution and expression. hnRNP A2/B1 could be one of the target proteins of HMBA, who cooperates with other regulators such as Actin and Prohibitin to regulate proliferation and differentiation. Further investigations on the function of hnRNP A2/B1 in the nuclear matrix and the interaction of hnRNP A2/B1 with related proteins need to be carried out.

Acknowledgements

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References

[1] Kosturko L D, Maggipinto M J, Korza G, et al. Heterogeneous nuclear ribonucleoprotein (hnRNP) E1 binds to hnRNP A2 and inhibits translation of A2 response element mRNAs [J]. Mol Biol Cell, 2006,17(8):3521-3533.

[2] Moran-Jones K, Wayman L, Kennedy D D, et al. hnRNP A2, a potential ssDNA/RNA molecular adapter at the telomere [J]. Nucleic Acids Res, 2005,33(2):486-496.

[3] Griffin M E, Hamilton B J, Roy K M, et al. Post-transcriptional regulation of glucose transporter-1 by an AU-rich element in the 3'UTR and by hnRNP A2 [J]. Biochem Biophys Res Commun, 2004,318(4):977-982.

[4] Hutchison S, LeBel C, Blanchette M, et al. Distinct sets of adjacent heterogeneous nuclear ribonucleoprotein (hnRNP) A1/A2 binding sites control 5' splice site selection in the hnRNP A1 mRNA precursor [J]. J Biol Chem, 2002,277(33):29745-29752.

[5] Zech V F, Dlaska M, Tzankov A, et al. Prognostic and diagnostic relevance of hnRNP A2/B1, hnRNP B1 and S100 A2 in non-small cell lung cancer [J]. Cancer Detect Prev, 2006,30(5):395-402.

[6] Fritsch-Stork R, Mullegger D, Skriner K, et al. The spliceosomal autoantigen heterogeneous nuclear ribonucleoprotein A2 (hnRNP-A2) is a major T cell autoantigen in patients with systemic lupus erythematosus [J]. Arthritis Res Ther, 2006,8(4):R118.

[7] Maggipinto M, Rabiner C, Kidd G J, et al. Increased expression of the MBP mRNA binding protein HnRNP A2 during oligodendrocyte differentiation [J]. J Neurosci Res, 2004,75(5):614-623.

[8] Zhou J, Allred DC, Avis I, et al. Differential expression of the early lung cancer detection marker, heterogeneous nuclear ribonucleoprotein-A2/B1 (hnRNP-A2/B1) in normal breast and neoplastic breast cancer [J]. Breast Cancer Res Treat, 2001, 66(3):217-224.

[9] Zhao C H, Li Q F, Zhao Y, et al. Changes of nuclear matrix proteins following the differentiation of human osteosarcoma MG-63 cells [J]. Genomics Proteomics Bioinformatics, 2006,4(1):10-17.

[10] Zhao C H, Li Q F. Altered profiles of nuclear matrix proteins during the differentiation of human gastric mucous adenocarcinoma MGc80-3 cells [J]. World J Gastroenterol, 2005,11(30): 4628-4633.

[11] Li Q F, Ma Z Y, Tang J. The effects of HMBA on morphology and expression of terminal differentiation markers in human osteosarcoma MG-63 cell [J]. Journal of Xiamen University (Natural Science), 2007,46(6): 836-841. (in Chinese)

[12] Ma Z Y, Li Q F, Tang J. The effects of HMBA on proliferation and associated genes expression of human osteosarcoma MG-63 cells [J]. Journal of Xiamen University (Natural Science), 2007,46(5):702-706. (in Chinese)

[13] Li Q F. Effect of retinoic acid on the changes of nuclear matrix in termediate filament system in gastric carcinoma cells [J]. World J Gastroenterol, 1999,5:417-420

[14] Michishita E, Kurahashi T, Suzuki T, et al. Changes in nuclear matrix proteins during the senescence- like phenomenon induced by 5-chlorodeoxyuridine in HeLa cells [J]. Exp Gerontol, 2002,37:885-890.

[15] Zhou J, Nong L, Wloch M, et al. Expression of early lung cancer detection marker: hnRNP-A2/B1 and its relation to microsatellite alteration in non-small cell lung cancer [J]. Lung Cancer, 2001,34(3): 341-350.

[16] Satoh H, Kamma H, Ishikawa H, et al. Expression of hnRNP A2/B1 proteins in human cancer cell lines [J]. Int J Oncol, 2000,16(4):763-767.

[17] Lee C H, Lum J H, Cheung B P, et al. Identification of the heterogeneous nuclear ribonucleoprotein A2/B1 as the antigen for the gastrointestinal cancer specific monoclonal antibody MG7 [J]. Proteomics, 2005,5(4):1160-1166.

[18] Eggert M, Michel J, Schneider S, et al. The glucocorticoid receptor is associated with the RNA-binding nuclear matrix protein hnRNP U [J].J Biol Chem, 1997,272(45):28471-28478.

[19] Alberti I, Barboro P, Barbesino M, et al. Changes in the expression of cytokeratins and nuclear matrix proteins are correlated with the level of differentiation in human prostate cancer [J]. J Cell Biochem, 2000,79:471-485.

[20] Jin M-L, Zhang P, Ding M-X, et al. Altered expression of nuclear matrix proteins in etoposide induced apoptosis in HL-60 cells [J]. Cell Res, 2001,11:125-134.

[21] Kadam K, D'souza S, Natraj U. Spatial distribution of actin and tubulin in human sperm nuclear matrix-intermediate filament whole mounts-a new paradigm [J]. Microsc Res Tech, 2007,70(7):589-598.

[22] Bohnsack M T, Stuven T, Kuhn C, et al. A selective block of nuclear actin export stabilizes the giant nuclei of xenopus oocytes [J]. Nat Cell Biol, 2006,8(3):257-263.

[23] Prasad S, Soldatenkov V A, Srinivasarao G, et al. Intermediate filament proteins during carcinogenesis and apoptosis [J]. Int J Oncol, 1999,14(3):563-570.

[24] Mishra S, Murphy L C, Nyomba B L, et al. Prohibitin: a potential target for new therapeutics [J]. Trends Mol Med, 2005,11(4):192-197.

[25] Wang S, Fusaro G, Padmanabhan J, Chellappan S-P. Prohibitin co-localizes with Rb in the nucleus and recruits N-CoR and HDAC1 for transcriptional repression [J]. Oncogene, 2002,21(55):8388-8396.