Arch. Biol. Sci., Belgrade, 65 (3), 929-937, 2013 DOI:10.2298/ABS1303929K

NIR IS A NUCLEOLAR RNA-BINDING PROTEIN THAT IS INVOLVED IN CELL PROLIFERATION

MIN KOOK KANG and SEUNG JIN HAN*

Department of Biological Sciences, Inje University, Gimhae, Gyeongnam, 621-749, Republic of Korea

Abstract – A novel inhibitor of histone acetyltransferase repressor (NIR) is a histone acetylation inhibitor that associates with p53, p63 and histone complexes. Staining with anti-NIR antibodies revealed that the protein is localized at the nucleolus. The nucleolar localization of NIR was lost upon RNase treatment, whereas its localization was unaffected by treatment with DNase. These results indicate that NIR directly or indirectly binds RNA in the nucleolus. The expression of NIR protein was higher in cancer cell lines than in non-cancer cell lines. The proliferation rate of human embryonic kidney 293T cells was higher following the ectopic expression of NIR than following the ectopic expression of green fluorescent protein (GFP). In summary, NIR is an RNA-associated protein that is involved in the cell proliferation regulation.

Key words: Cell cycle, cell proliferation, NIR, nucleolus, RNA-binding protein

INTRODUCTION

Initially, the nucleolus was simply thought to be the site of ribosomal RNA (rRNA) transcription and processing that is required to synthesize the ribosome complex. However, several studies suggest that the nucleolus also plays pivotal roles in the processing of telomere RNA, transfer RNA (tRNA), U6 small nuclear RNA and signal recognition particle (SRP) RNA (Pederson, 1998). Furthermore, the nucleolus is involved in gene expression, cell cycle regulation and DNA damage repair (Emmott and Hiscox, 2009). p53 and its regulator, MDM2, are important factors in these processes (Klibanov et al., 2001). Protein 53 is a transcription factor for several cell cycle arrest genes, such as *p21*, and several pro-apoptotic genes, such as *BAX*, *PIG3*, and *NOXA* (Riley et al., 2008). The stability of the p53 protein is regulated by ubiquitination that is conducted by the p53-specific ubiquitin ligase, MDM2. The nucleolus is the place where MDM2 is sequestered by binding to ARF after cellular stress, such as ultraviolet irradiation and DNA damage, which thereby leads to p53 stabilization (Weber et al., 1999).

The novel inhibitor of histone acetyltransferase repressor (NIR) was isolated as a binding partner of p53 (Heyne et al., 2010, Hublitz et al., 2005). It also binds core histones and thereby prevents the acetylation of histones by histone acetyltransferases in p53-dependent reporter genes and endogenous p53 target genes (Heyne et al., 2010, Hublitz et al., 2005). Depletion of NIR using short interfering RNA increases p53-dependent apoptosis (Hublitz et al., 2005). NIR promotes phosphorylation of the DNA-binding domain of p53 by Aurora B kinase, thereby inhibiting transcription of p53 target genes (Wu et al., 2011). NIR also binds p63, a major regulator of stratified epithelial morphogenesis, and inhibits p63-dependent transactivation of *p21* (Heyne et al.,

2010). NIR has recently been reported to play a role in the rRNA processing of the 40S and 60S ribosomal subunits (Wu et al., 2012).

To understand better the roles of NIR in cell cycle regulation, we studied the cellular localization of NIR and examined the rate of cell proliferation after overexpression of NIR. NIR is localized in the nucleolus by binding to RNA or an RNA-binding protein and is involved in cell proliferation.

MATERIALS AND METHODS

Construction of NIR expression plasmids

Human NIR cDNA was subcloned between the *Kpn*I and *Not*I sites of pcDNA3.1, and was tagged with a FLAG epitope at the carboxyl terminus. The resulting plasmid was digested with *Kpn*I and *Eco*RV and the fragment was subcloned into the pEGFP-C1 vector that had been digested with *Kpn*I and *Sma*I to generate amino terminally GFP-tagged NIR.

Production of anti-NIR antibodies

A fragment of mouse NIR cDNA (amino acids 500–750) was digested with *Sac*I and *Xho*I and inserted into the pQE31 vector to allow expression of His-tagged NIR. The protein was expressed, purified and then injected into rabbits to raise a polyclonal antibody against mouse NIR (MoAb). A polyclonal antibody against human NIR (HuAb) was raised in rabbits using a 17 amino acid peptide of the carboxyl terminus of human NIR (AQGPEDELEDLQLSEDD) (Peptron, Deajeon, Korea). Both antibodies were purified and used for Western blotting and immunocytochemistry.

Cell culture and cell transfection

Human embryonic kidney (HEK) 293T, HeLa and NIH3T3 cells were cultured in Dulbecco modified Eagle's medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (FBS, Life technologies, Carlsbad, CA), 100 IU/ml penicillin and 100 μg/ml streptomycin at 37°C in 5% CO_2. For ectopic expression of NIR constructs, cells were grown to 70% confluency on 60 mm or 100 mm dishes and were then transfected using the JetPEI transfection reagent (Polyplus Transfection Inc, NY), according to the manufacturer's protocol. All of the cell lines, including non-cancer cell lines (WI-38, lung fibroblast; MRC5, lung fibroblast; Hel 299, lung embryo fibroblast) and cancer cell lines (A427, lung carcinoma; A549, lung carcinoma; NCI-H23, non-small cell lung adenocarcinoma), were purchased from the Korean Cell Line Bank (Seoul, Korea). To check the cell growth rate after NIR transfection, the number of cells was counted with a hemocytometer after trypsinization. To synchronize HeLa cell at early S phase, aphidicolin, a reversible inhibitor of eukaryotic nuclear DNA replication, was used. 8×10^5 HeLa cells were plated in 60-mm dishes, and aphidicolin was added to a final concentration of 5nM for 12 h. The synchronized cells were collected every 3 h after release from early S block by removing the reagent.

Western blot analysis

Cells were cultured for 24 h after transfection and then washed with cold phosphate buffered saline (PBS). For cell fractionation, whole-cell extracts were prepared in RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 1 mM PMSF, 50 mM NaF, 4 μg/ml aprotinin, 0.7 μg/ml pepstatin and 0.5 μg/ml leupeptin), which enables protein extraction from cytoplasmic, membrane and nuclear compartments. The sample was rotated at 4°C for 15 min and centrifuged at 13,000 rpm for 20 min. The supernatant containing soluble proteins was collected (soluble fraction, S). The pellet was resuspended in the same volume of SDS-polyacrylamide gel electrophoresis (PAGE) loading buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.1 M DTT, 0.005% bromophenol blue) and was boiled to extract proteins (insoluble fraction, I). The same amount of soluble or insoluble cell lysates was subjected to SDS-PAGE and Western blotting. NIR was detected by incubating the blot for 2 h with purified anti-NIR antibodies (1:1000 dilution), and then with horseradish peroxidase conjugated anti-rabbit IgG (Jack-

son ImmunoResearch, West Grove, PA). Proteins were visualized with enhanced chemiluminescence (GE Healthcare Bio-Sciences).

Immunocytochemistry

To examine the cellular localization of NIR, cells were grown on poly-L lysine-coated coverslips. After fixation with 4% formaldehyde prepared in PBS for 1 hr, cells were permeabilized with blocking solution (PBS containing 1% BSA and 0.3% Triton X-100) for 20 min on ice. Cells were incubated with anti-NIR antibodies prepared in blocking solution for 2 h and were then incubated with an FITC- or Cy3-conjugated anti-rabbit secondary antibody prepared in blocking solution for 20 min. DNA was stained with Hoechst or DAPI solutions (Invitrogen, Carlsbad, California). Mounted coverslips were observed with a Nikon Eclipse 2000 microscope (Melville, NY). To determine the effects of DNase and RNase on the localization of NIR, we modified a previously reported protocol (Galcheva-Gargova et al., 1998). Briefly, HeLa cells grown on coverslips were permeabilized with 0.1% Triton X-100 for 3 min on ice. Cells were washed three times, and were then incubated for 60 min at 37°C with PBS containing 0.1 mg/ml RNase A or PBS containing 0.1 mg/ml DNase I and 5 mM $MgCl_2$. Cells were incubated with PBS as a negative control.

RESULTS

Characterization of anti-NIR antibodies

In order to determine the role of the NIR in the cell cycle, we raised polyclonal antibodies against a fragment of mouse NIR (amino acids 500-750) and a 17-amino-acid peptide from the carboxyl terminus of human NIR. Human NIR consists of 749 amino acids (750 amino acids for mouse), therefore, the calculated molecular weight of NIR protein is about 85kD. The antibody which was generated with human NIR peptide recognized endogenous and overexpressed NIR protein by Western blotting around 95kD (Fig. 1A). Endogenous and exogenous NIR protein in the insoluble fraction migrated slower than that in the soluble fraction (Fig. 1A). The MoAb also generated an intense band corresponding to NIR in various adult mouse tissues, including the brain, kidney, lung, thymus, heart and liver (Fig. 1B). These results indicate that the generated antibodies can specifically recognize NIR protein and that NIR protein is widely expressed in various murine tissues.

NIR accumulates in the nucleolus

The cellular localization of NIR is controversial. NIR localizes in the nucleoplasm in doxorubicin-treated BHK cells (Hublitz et al., 2005) and in U2OS cells overexpressing Aurora B kinase (Wu et al., 2011). In contrast, NIR is reported to localize in the nucleolus in U2OS cells and HeLa cells (Wu et al., 2012). NIR is found in the nucleolus and nucleoplasm in H1299 cells transfected with TAp63γ (Heyne et al., 2010). Therefore, immunocytochemistry with the two anti-NIR antibodies was performed to study the cellular localization of endogenous NIR. Fluorescence was detected predominantly in the nucleolus in almost all cells, indicating that NIR is localized in the nucleolus (Fig. 2). Both antibodies had the same fluorescence pattern in human HeLa cells (Fig 2B.g) and mouse NIH3T3 cells (Fig. 2C.j). Fluorescence was not detected when cells were treated with the secondary antibody alone (Fig 2A.c), indicating that the fluorescence generated was due to the anti-NIR antibodies. To confirm that NIR localizes in the nucleolus, we overexpressed GFP-NIR in HeLa cells. GFP fluorescence was mainly detected in the nucleolus and a faint signal was detected in the nucleoplasm (Fig 2D.n). These results indicate that endogenous NIR is predominantly located in the nucleolus in resting cells and that overexpression of the protein could lead to translocation of NIR to nucleoplasm.

RNA is required for the nucleolar localization of NIR

The nucleolus contains abundant ribosomal DNA and is the site at which various types of RNA are processed, including rRNA, telomere RNA, tRNA, U6 small nuclear RNA and SRP RNA. Consequently, we examined whether the nucleolar localization of NIR requires DNA and/or RNA by treating cells with

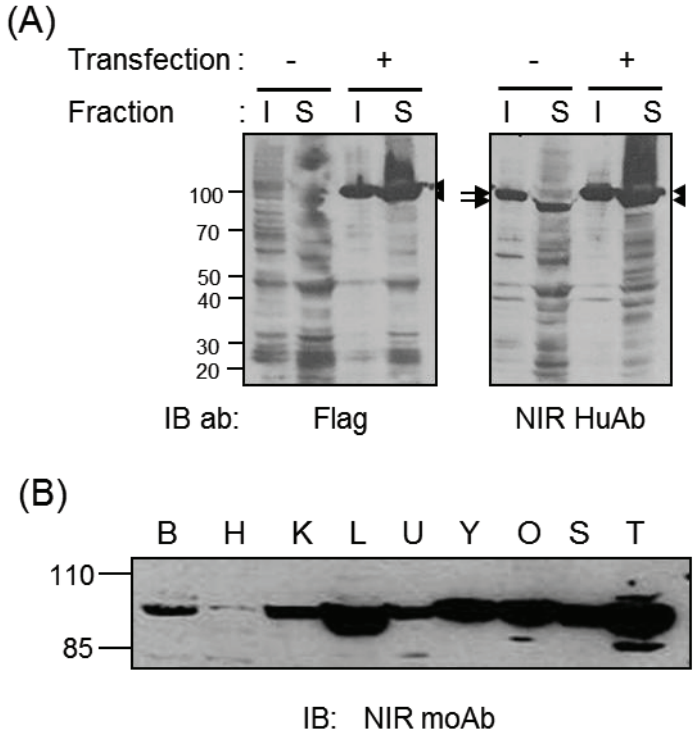

Fig. 1. NIR is widely expressed in various murine tissues. (A) Flag-NIR transfected (+) or non-transfected (-) HEK293 cell lysates were detected with Flag antibody (left panel). The same blot was stripped and re-probed with NIR HuAb (right panel). HuAb detected endogenous NIR (arrows) and FLAG-tagged NIR (arrowheads). FLAG-tagged NIR migrated slower than endogenous NIR due to the additional 15 amino acid residues of the tag. NIR in the soluble fraction (S) migrated faster than NIR in the insoluble fraction (I). (B) The expression of NIR in various murine tissues was examined by Western blotting. Lysates of various tissues were prepared and fractionated on an 8% SDS-PAGE gel (100 μg per lane). The gel was transferred to a PVDF membrane and probed with MoAb. The positions of protein standards are indicated on the left (in kDa). B, brain; H, heart; K, kidney; L, liver; U, lung; Y, lymph node; O, ovary; S, spleen; T, thymus.

DNase or RNase, respectively. NIR was no longer localized at the nucleolus following RNase treatment, whereas its localization was unaffected by DNase treatment (Fig 3). Staining with Hoechst solution was not detected when cells were treated with DNase, indicating that the treatment effectively cleaved DNA. These results indicate that the nucleolar localization of NIR is dependent on its association with RNA or an RNA-bound protein.

NIR expression is modulated throughout the cell cycle

NIR interacts with p53 and p63, which regulate cell cycle arrest (Heyne et al., 2010, Hublitz et al., 2005). Consequently, we investigated whether NIR expression changes throughout the cell cycle in cell cycle arrested and released HeLa cells. As shown in the Fig. 4A, NIR expression decreased as cell enters M phase though G2. Since NIR expression appears to depend on cell cycle progression, NIR protein expression was examined in several non-cancer cell lines (WI-38, MRC5, Hel 299) and several cancer cell lines (A427, A549, NCI-H23). Generally, NIR expression was higher in the cancer cell lines than in the non-cancer cell lines (Fig. 4B). The level of NIR was similar in the insoluble fraction of each of the cell lines, whereas NIR expression was markedly higher in the soluble fractions of the cancer cell lines than in those of the non-cancer cell lines. β-tubulin was used as a loading control. These results suggest that the expression of NIR is modulated throughout the cell cycle.

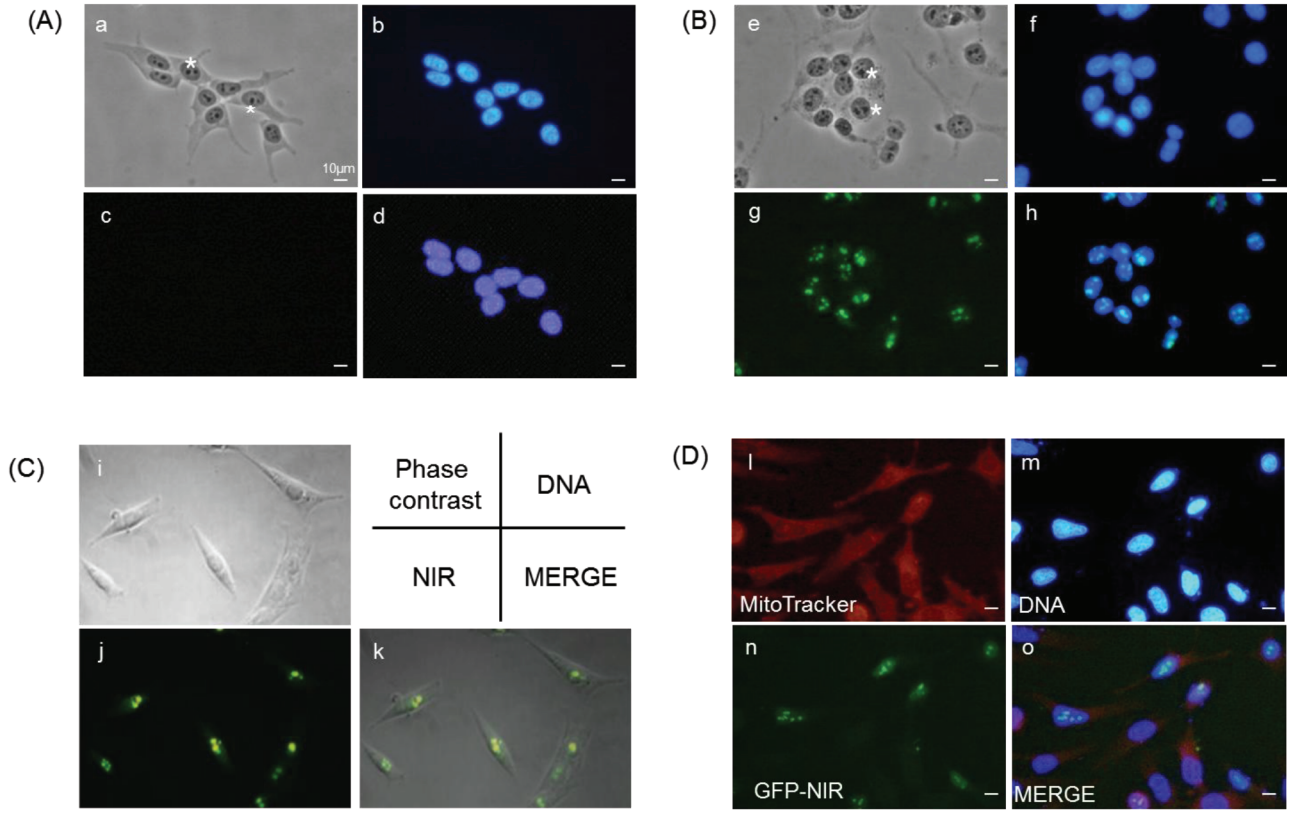

Fig. 2. NIR accumulates in the nucleolus. (A) Cells were treated with a FITC-conjugated anti-rabbit antibody alone to confirm that the signals in (g) and (j) are due to NIR specific Abs (a–d). The asterisked dark structures in the nucleus are the nucleoli (a, e). (scale bar, 10 μm) (B) Endogenous NIR was detected using HuAb and a FITC-conjugated anti-rabbit antibody (e–h). HeLa cells grown on coverslips were stained with HuAb and a FITC-conjugated anti-rabbit antibody. The localization of endogenous NIR in HeLa cells is predominantly nucleolar. (C) MoAb specifically labels the NIR in the nucleolus of NIH3T3 cells same as HuAb in (B). (D) GFP-NIR was primarily localized in the nucleolus, similar to endogenous NIR. GFP-NIR was also detected in the nucleoplasm. Mitochondria were stained with Mitotracker® for 30 min prior to fixation to distinguish between the cytoplasm and nuclei (l). Nuclei were stained with Hoechst (b, f, m).

Overexpression of NIR enhances cell proliferation

NIR expression varied throughout the cell cycle and NIR may be involved in cell cycle arrest via its association with p53 and p63. Therefore, the effect of NIR on cell proliferation was investigated by overexpressing HA- or GFP-tagged NIR in HeLa cells. To exclude the effect of ectopic protein expression on the cell growth, GFP was used as a negative control. The number of HeLa cells was 2-fold higher 4 days after transfection of HA-NIR or GFP-NIR than after transfection of GFP (Fig. 5). These results suggest that NIR regulates cell proliferation.

DISCUSSION

Many lines of evidence indicate that the nucleolus is not only the site of ribosome biogenesis but is also responsible for the regulation of many cellular processes, such as the cell cycle, apoptosis and stress sensing (Montanaro et al., 2008, Sirri et al., 2008). Proteomic analyses suggest there are more than 270 proteins in the nucleolus (Andersen et al., 2002). Among them, B23 (also known as nucleophosmin) and C23 (also known as nucleolin) have been well studied. These proteins interact with nucleic acids and are involved in ribosome biogenesis (Cong et al., 2012, Okuwaki et al., 2002). However, several recent

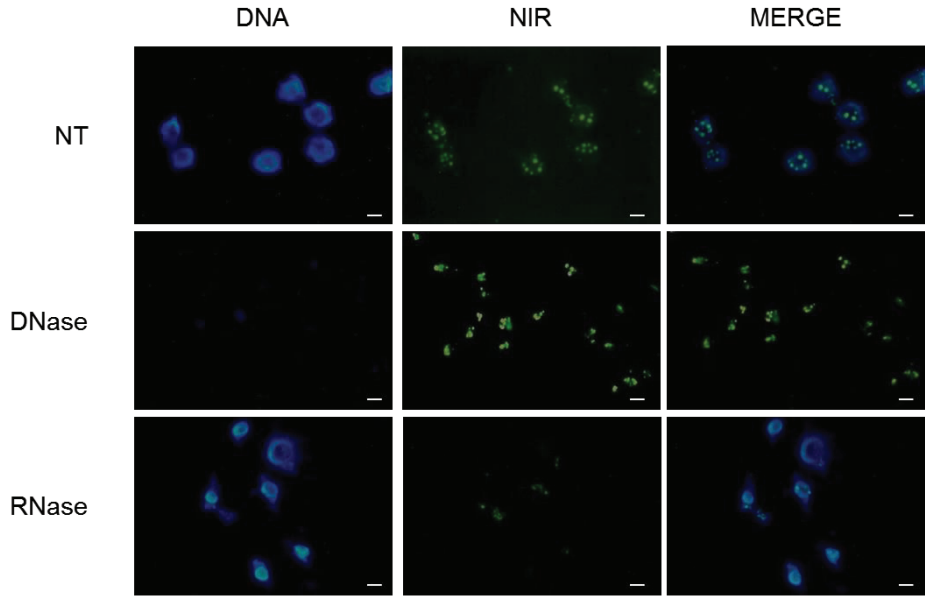

Fig. 3. RNA is required for the nucleolar localization of NIR. NIH3T3 cells were treated with DNase or RNase as described in the materials and methods. After fixation, NIR antibody (MoAb) and a FITC-conjugated anti-rabbit antibody. Cells were incubated with PBS as a negative control (not treated, NT). Nuclear DNA was stained with DAPI.

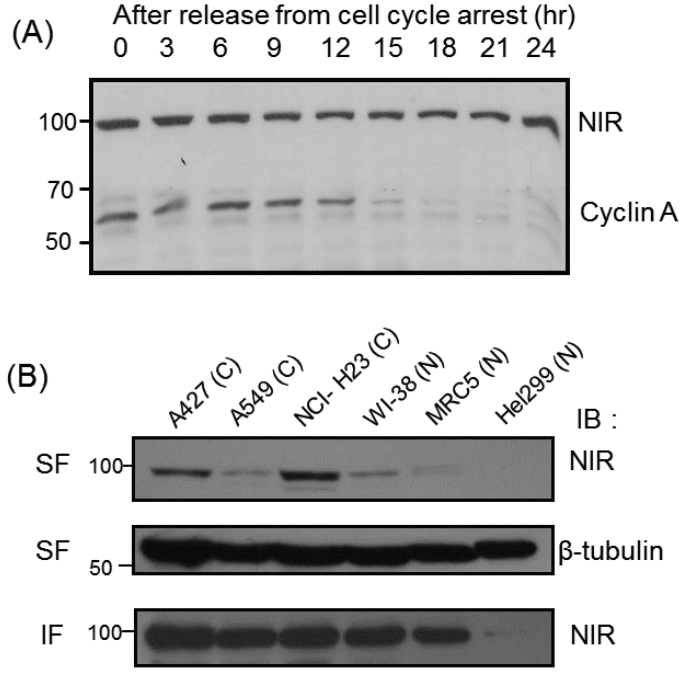

Fig. 4. NIR expression is modulated throughout the cell cycle. (A) NIR expression decreased during M phase. HeLa cells were harvested at various time-points after being released from cell cycle arrest which was induced by aphidicolin treatment. Cell lysates were resolved by SDS-PAGE, and Western blotting was performed with MoAb. Cyclin A which is mainly expressed in the G_2 phase was monitored as a cell cycle indicator. The positions of protein standard markers are indicated on the left (in kDa). (B) NIR expression was monitored in three cancer cell lines (C) and three non-cancer cell lines (N). Generally, NIR expression in the soluble fraction was higher in cancer cell lines than in non-cancer cell lines. β-tubulin was used as a loading control.

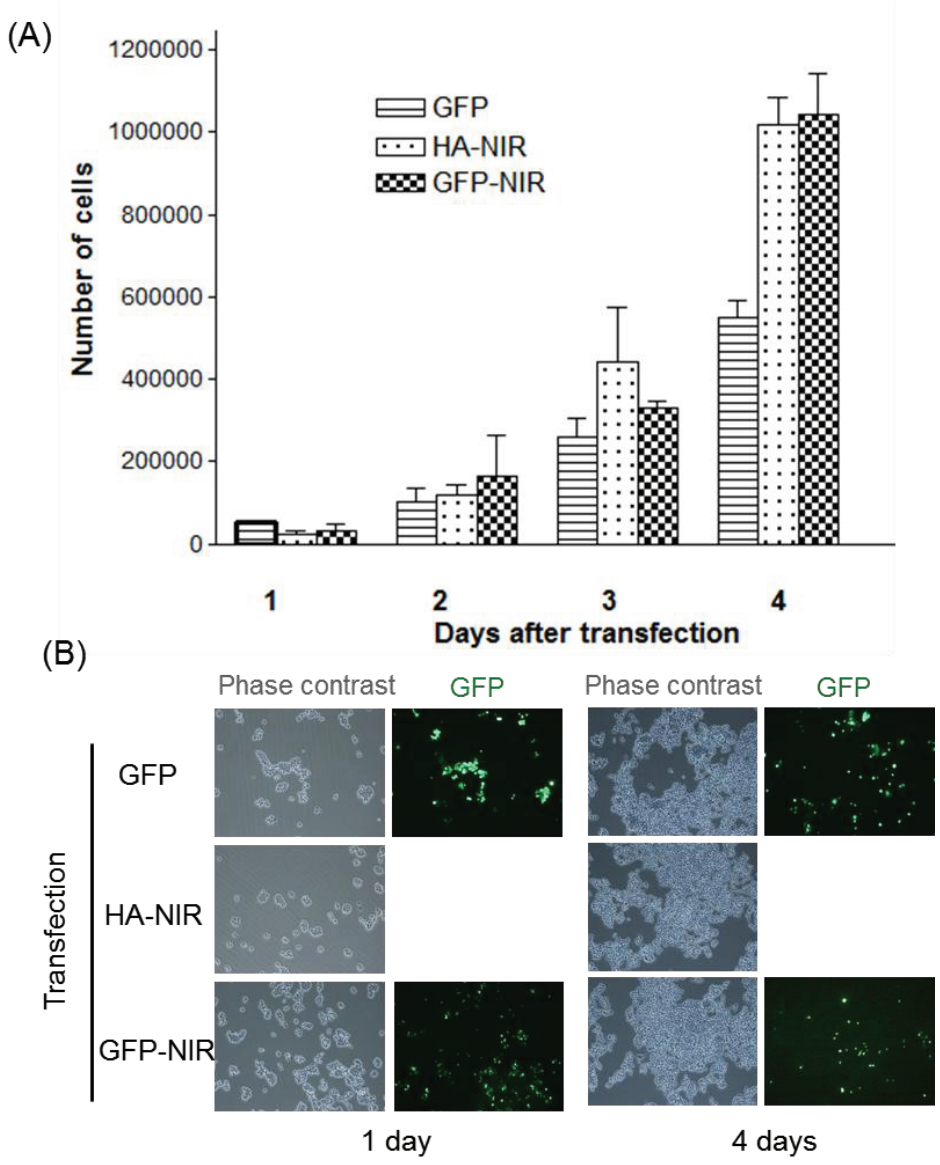

Fig. 5. Overexpression of NIR enhances cell proliferation. (A) HEK293 cells ($1x10^5$) cultured on 60 mm dishes in media containing 10% FBS were transfected with GFP, HA-NIR or GFP-NIR. Cells were counted 1, 2, 3, and 4 days after transfection to determine the level of cell proliferation. The number of cells was significantly higher 4 days after transfection of HA-NIR or GFP-NIR than after transfection of GFP. **(B)** The expression of transfected gene was monitored with fluorescence.

reports suggest that B23 and C23 also contribute to the regulation of the cell cycle, cell proliferation, and centrosome duplication, since genomic instability and defective centrosome duplication are observed when these proteins are inactivated (Okuda et al., 2000, Reboutier et al., 2012, Tarapore et al., 2002, Ugrinova et al., 2007). Another nucleolar protein, nucleolar protein p120 (NOL1), binds rRNA and its expression increases during proliferation. Overexpression of NOL1 leads to the malignant transformation of NIH3T3 cells and NOL1 expression dramatically increases during the G1/S transition, which suggests that NOL1 regulates the cell cycle (Fonagy et al., 1993). These data indicate that many proteins

that regulate cell proliferation reside within the nucleolus. The exact role of NIR, a nucleolar protein (Fig. 2), remains unclear. NIR expression was higher in cancer cell lines than in non-cancer cell lines (Fig. 4B) and the level of NIR fluctuated during the cell cycle progression (Fig. 4A). Furthermore, the rate of cell proliferation increased following overexpression of NIR (Fig. 5). The ability of NIR to regulate cell proliferation may be dependent on its interaction with p53 and p63, which regulate cell cycle arrest and apoptosis. However, is it also possible that the effects of NIR upon cell proliferation are independent of p53 and p63. Further study is required to determine whether NIR modulates cell proliferation by binding another protein(s).

Since NIR has a putative nuclear localization signal, we decided to study the localization of NIR protein using two approaches. First, anti-NIR antibodies were used to determine the localization of endogenous NIR protein. Second, the localization of overexpressed GFP-NIR was observed. In both cases, NIR accumulated in the nucleolus and a small portion of the protein was detected in the nucleoplasm (Fig 2), in agreement with a previous study (Heyne et al., 2010). However, this result is inconsistent with other studies that reported that, according to immunocytochemical analyses, NIR is mainly localized in the nucleoplasm (Hublitz et al., 2005, Wu et al., 2011). It is possible that specific signals or conditions stimulate translocation of NIR from the nucleolus to the nucleoplasm. Many nucleolar proteins can be redistributed to other locations. B23 shuttles between the cytoplasm and the nucleolus by binding other nucleolar proteins (Ma et al., 2006, Yung et al., 1991). C23 is located in the nucleolus, nucleoplasm and at the cell surface (Masiuk et al., 2007, Xue et al., 1993). Because NIR, C23 and B23 are all predominantly localized in the nucleolus and shuttle to another location, it is possible that NIR interacts with these proteins. Indeed, C23 and B23 interact each other (Li et al., 1996).

The calculated molecular weight of NIR is about 85kD; however, NIR was detected around 95kD in the SDS-PAGE gel (Fig 1). In addition, NIR migrated faster in the soluble fraction (nucleoplasm and cytoplasm) than in the insoluble fraction (nucleolus) (Fig. 1A). This suggests that a post-translational modification of NIR, such as dephosphorylation, deacetylation, desumoylation or deubiquitination, is responsible for its redistribution from the nucleolus to the nucleoplasm. Aurora B kinase interacts with the complex of NIR and p53, even though the kinase phosphorylates p53 not NIR (Wu et al., 2011). It is possible that another kinase was responsible for NIR phosphorylation. In fact, phosphor-amino acid analysis indicated that a serine residue(s) of NIR is phosphorylated in our recent study (data not shown). Further study is required to characterize this modification, which may provide insight into the function of NIR. Cellular proliferation is controlled by a complex network of signaling molecules and further investigation is warranted to determine how this signaling controls the function of NIR.

Acknowledgments - This work was supported by an Inje University Research Grant.

REFERENCES

Andersen, J.S., Lyon, C.E., Fox, A.H., Leung, A.K., Lam, Y.W., Steen, H., Mann, M. and *A.I. Lamond* (2002). Directed proteomic analysis of the human nucleolus. *Curr Biol* **12,** 1-11.

Cong, R., Das, S., Ugrinova, I., Kumar, S., Mongelard, F., Wong, J. and *P. Bouvet* (2012). Interaction of nucleolin with ribosomal RNA genes and its role in RNA polymerase I transcription. *Nucleic Acids Res.*

Emmott, E. and *J.A. Hiscox* (2009). Nucleolar targeting: the hub of the matter. *EMBO Rep* **10,** 231-238.

Fonagy, A., Swiderski, C., Wilson, A., Bolton, W., Kenyon, N. and *J.W. Freeman* (1993). Cell cycle regulated expression of nucleolar antigen P120 in normal and transformed human fibroblasts. *J Cell Physiol* **154,** 16-27.

Galcheva-Gargova, Z., Gangwani, L., Konstantinov, K.N., Mikrut, M., Theroux, S.J., Enoch, T. and *R.J. Davis* (1998). The cytoplasmic zinc finger protein ZPR1 accumulates in the nucleolus of proliferating cells. *Mol Biol Cell* **9,** 2963-2971.

Heyne, K., Willnecker, V., Schneider, J., Conrad, M., Raulf, N., Schule, R. and *K. Roemer* (2010). NIR, an inhibitor of histone acetyltransferases, regulates transcription factor TAp63 and is controlled by the cell cycle. *Nucleic Acids Res.*

Hublitz, P., Kunowska, N., Mayer, U.P., Muller, J.M., Heyne, K., Yin, N., Fritzsche, C., Poli, C., Miguet, L., Schupp, I.W., van Grunsven, L.A., Potiers, N., van Dorsselaer, A., Metzger, E., Roemer, K. and *R. Schule* (2005). NIR is a novel INHAT repressor that modulates the transcriptional activity of p53. *Genes Dev* **19,** 2912-2924.

Klibanov, S.A., O'Hagan, H.M. and *M. Ljungman* (2001). Accumulation of soluble and nucleolar-associated p53 proteins following cellular stress. *J Cell Sci* **114,** 1867-1873.

Li, Y.P., Busch, R.K., Valdez, B.C. and *H. Busch* (1996). C23 interacts with B23, a putative nucleolar-localization-signal-binding protein. *Eur J Biochem* **237,** 153-158.

Ma, Z., Kanai, M., Kawamura, K., Kaibuchi, K., Ye, K. and *K. Fukasawa* (2006). Interaction between ROCK II and nucleophosmin/B23 in the regulation of centrosome duplication. *Mol Cell Biol* **26,** 9016-9034.

Masiuk, M., Urasinska, E. and *W. Domagala* (2007). Intranuclear nucleolin distribution during cell cycle progression in human invasive ductal breast carcinomas in relation to estrogen receptor status. *Anticancer Res* **27,** 3957-3962.

Montanaro, L., Trere, D. and *M. Derenzini* (2008). Nucleolus, ribosomes, and cancer. *Am J Pathol* **173,** 301-310.

Okuda, M., Horn, H.F., Tarapore, P., Tokuyama, Y., Smulian, A.G., Chan, P.K., Knudsen, E.S., Hofmann, I.A., Snyder, J.D., Bove, K.E. and *K. Fukasawa* (2000). Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. *Cell* **103,** 127-140.

Okuwaki, M., Tsujimoto, M. and *K. Nagata* (2002). The RNA binding activity of a ribosome biogenesis factor, nucleophosmin/B23, is modulated by phosphorylation with a cell cycle-dependent kinase and by association with its subtype. *Mol Biol Cell* **13,** 2016-2030.

Pederson, T. (1998). The plurifunctional nucleolus. *Nucleic Acids Res* **26,** 3871-3876.

Reboutier, D., Troadec M.B., Cremet J.Y., Fukasawa K. and *C. Prigent* (2012). Nucleophosmin/B23 activates Aurora A at the centrosome through phosphorylation of serine 89. *J Cell Biol* **197,** 19-26.

Riley, T., Sontag, E., Chen, P. and *A. Levine* (2008). Transcriptional control of human p53-regulated genes. *Nat Rev Mol Cell Biol* **9,** 402-412.

Sirri, V., Urcuqui-Inchima, S., Roussel, P. and *D. Hernandez-Verdun* (2008). Nucleolus: the fascinating nuclear body. *Histochem Cell Biol* **129,** 13-31.

Tarapore, P., Okuda, M. and *K. Fukasawa* (2002). A mammalian in vitro centriole duplication system: evidence for involvement of CDK2/cyclin E and nucleophosmin/B23 in centrosome duplication. *Cell Cycle* **1,** 75-81.

Ugrinova, I., Monier, K., Ivaldi, C., Thiry, M., Storck, S., Mongelard, F. and *P. Bouvet* (2007). Inactivation of nucleolin leads to nucleolar disruption, cell cycle arrest and defects in centrosome duplication. *BMC Mol Biol* **8,** 66.

Weber, J.D., Taylor, L.J., Roussel, M.F., Sherr, C.J. and *D. Bar-Sagi* (1999). Nucleolar Arf sequesters Mdm2 and activates p53. *Nat Cell Biol* **1,** 20-26.

Wu, J., Zhang, Y., Wang, Y., Kong, R., Hu, L., Schuele, R., Du, X. and *Y. Ke* (2012). Transcriptional repressor NIR functions in the ribosome RNA processing of both 40S and 60S subunits. *PLoS One* **7,** e31692.

Wu, L., Ma, C.A., Zhao, Y. and *A. Jain* (2011). Aurora B interacts with NIR-p53, leading to p53 phosphorylation in its DNA-binding domain and subsequent functional suppression. *J Biol Chem* **286,** 2236-2244.

Xue, Z., Shan, X., Lapeyre, B. and *T. Melese* (1993). The amino terminus of mammalian nucleolin specifically recognizes SV40 T-antigen type nuclear localization sequences. *Eur J Cell Biol* **62,** 13-21.

Yung, B.Y., Yang, Y.H. and *A.M. Bor* (1991). Nucleolar protein B23 translocation after deferoxamine treatment in a human leukemia cell line. *Int J Cancer* **48,** 779-784.