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A Microtubule Associated Protein (hNUDC) Binds to the Extracellular domain of Thrombopoietin Receptor (Mpl)

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Abstract Human NUDC (hNUDC) was initially characterized as a nuclear migration protein based on the similarity of its C-terminus to that of fungal NUDC from *Aspergillus nidulans*. However, hNUDC is a 331 amino acid protein whereas fungal NUDC is 198 amino acids in length. The extra N-terminal portion of hNUDC has no known function or homology to other proteins. In this study, we report the binding of hNUDC to the extracellular domain of the thrombopoietin receptor (Mpl) as detected by the yeast two-hybrid system, GST pull-down, and co-immunoprecipitation. Our deletion analysis demonstrated that amino acids between positions 100 and 238 as the critical domain mediating the hNUDC and Mpl interactions as detected by the two-hybrid system and GST pull-down assay. Immunofluorescence staining of human megakaryocyte cells indicated that hNUDC and Mpl colocalized at all stages of megakaryocyte development. Substantial colocalization of hNUDC with microtubules was also detected around nuclei and elongated microtubular structures, especially in proplatelet extensions. J. Cell. Biochem. 96: 741–750, 2005.

Key words: hNUDC; TPO; megakaryocyte; microtubules; dynein

The production of platelets from cells of the megakaryocyte lineage is a complex process, which is regulated by many hematopoietic growth factors. Interleukin-3 (IL-3), stem cell factor (SCF), and granulocyte macrophage colony-stimulating factor (GM-CSF) mainly effect the proliferation of megakaryocytes from progenitor cells [Kaushansky et al., 1986; Tanaka et al., 1992; Brandt et al., 1994]. Others growth factor, including interleukin-6 (IL-6), interleukin-11 (IL-11), and leukemia inhibiting factor (LIF) show their action on megakaryocyte maturation [Ishibashi et al., 1989; Paul et al., 1990; Burstein et al., 1992]. One growth factor, thrombopoietin (TPO), is involved in both proliferation and differentiation of the megakaryocyte lineage and therefore is crucial for thrombopoiesis [Bartley et al., 1994; de Sauvage et al., 1994; Lok et al., 1994; Wendling et al., 1994]. However, TPO may not be required for the last steps of megakaryocyte maturation and platelet formation [de Sauvage et al., 1996; Nagahisa et al., 1996]. In TPO-deficient mice, there was an 85%–90% reduction in both the number of megakaryocytes in bone marrow and in the number of circulating platelets, however, enough normal platelets were retained to prevent spontaneous bleeding, suggesting that some other factors can compensate for the lack of TPO [de Sauvage et al., 1996; Bunting et al., 1997].

The intracellular signaling elicited by TPO is mediated by the thrombopoietin receptor (Mpl), a member of the cytokine receptor superfamily, characterized by two copies of the consensus cytokine receptor homologous (CRH) domain involved in cytokine binding [Alexander et al., 1995]. The mechanism by which TPO activates Mpl appears to be similar to that of other hematopoietic growth factors such as erythropoietin (EPO), growth hormone (GH), prolactin (PRL), and granulocyte colony-stimulating factor (G-CSF), which bind homodimers of their receptors [Alexander, 1999]. Most members of the cytokine receptor superfamily have only one

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CRH domain. However, the extracellular region of Mpl has two CRH domains but only the distal domain is involved in binding of TPO [Sabath et al., 1999], suggesting the possibility that another cytokine may be involved in binding with Mpl [Feese et al., 2004]. With these considerations in mind, we used a yeast two-hybrid system to screen a fetal liver cDNA library for alternative ligands of Mpl. Our study revealed that hNUDC interacts with the extracellular domain of Mpl.

NUDC was first found as a nuclear distribution protein that is active in nuclear migration and cytokinesis in *Aspergillus nidulans* [Osmani et al., 1990; Xiang et al., 1994]. Similar studies in human cells have also shown an involvement in cell mitotic spindle formation and cell proliferation [Zhang et al., 2002]. It is expressed in most tissues in human cells but expression levels are much higher in hematopoietic precursor cells compared to other human tissues, and it was reported to play a functional role in promoting hematopoietic cell growth [Miller et al., 1999]. Human NUDC has been localized with Lis1 and the dynein motor at the microtubule-organizing center in mitotic spindles [Aumais et al., 2003] and at the leading pole of migrating neurons [Aumais et al., 2001]. Cytoplasmic dynein has already been reported to be involved in the activation of platelet formation [Rothwell and Calvert, 1997].

The functional significance of the association between hNUDC amd Mpl is unknown because none has been previously reported. In this study, we performed a combination of biochemical and immunofluorescence microscopy as an initial approach to demonstrate directly the interactions between hNUDC and Mpl in vitro.

MATERIALS AND METHODS

Antibodies

A rabbit polyclonal antibody was raised against a recombinant protein of the extracellular domain of Mpl that was fused with the maltose-binding protein (MBP-Mpl-EC) as described previously [Zhang et al., 2004]. Mouse anti-β-tubulin monoclonal antibody, R-phycoerythrin (R-PE), and fluorescein isothiocyanate (FITC)-conjugated secondary antibodies against rabbit or mouse IgM + IgG and 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from BioLegend (San Diego, CA).

Yeast Two-Hybrid Screening

The extracellular domain of Mpl (amino acids 26–491) was used as the bait in a yeast-two hybrid screen of a human fetal liver cDNA library constructed in pB42AD (CLONTECH). The interaction capacity was assayed for β-galactosidase activity according to the method specified in the Clontech MATCHMAKER two-hybrid protocol.

Purification of rhNUDC and Production of Antibodies Against hNUDC

The coding region of hNUDC was constructed in the pET28b vector (Invitrogen) downstream and in frame with a His-tag to give pET-hNUDC. *E. coli* strain BL21 (DE3) carrying pET-hNUDC was induced with 0.5 mM IPTG for 3 h at 30°C. The soluble fraction containing the recombinant hNUDC (rhNUDC) fusion protein was purified through a cobalt-based immobilized metal affinity chromatography (Co^{2+}IMAC) column (CLONTECH).

To generate polyclonal anti-hNUDC antibodies, we diluted 100 μg of the purified rhNUDC with an equal volume of complete Freund's adjuvant (first injection) or incomplete Freund's adjuvant (subsequent injections), and injected it subcutaneously into female New Zealand White rabbits every 3 weeks for a total of six injections per animal. The IgG fraction was purified from this antiserum using Affinity Pak™ protein A column (Amersham Biosciences).

To generate monoclonal anti-hNUDC antibodies, female BALB/c mice were immunized intraperitoneally with 20 μg of purified rhNUDC mixed with Freund's complete or incomplete adjuvant for the first and the three following injections, respectively. Injections were given at 2-week intervals, followed by an intravenous dose of the antigen without adjuvant 3 days prior to the fusion experiment. Splenic cells were fused with mouse myeloma Sp2/0-Ag14 cells using polyethylene glycol 4000 and hybrid cells were selected in Iscove's medium containing 10% fetal calf serum, G-418, sodium pyruvate, L-glutamine, hypoxanthine, and thymidine. Supernatants of hybrid cultures were screened by ELISA, and the reactions were confirmed with Western blot analysis. Positive clones were clonally selected twice by limiting dilution. During limiting dilution, only one subclone that stably secreted

monoclonal antibody was obtained. The secreted antibody from culture was purified using either Protein-A Sepharose CL-4B column or MABTrap Kit (Amersham Biosciences).

Construction and Purification of GST-Mpl-EC Fusion Protein

The gene sequence of the extracellular domain of Mpl (Mpl-EC) comprising amino acids from 26 to 491 were amplified by PCR and cloned into the *Escherichia coli* expression vector, pGEX4T-1, in frame with an N-terminal GST (Amersham Biosciences). The recombinant plasmid was transformed into *E. coli* strain BL21 for protein expression. The soluble fraction was incubated with Glutathione-Sepharose 4B (Amersham Biosciences) overnight at 4°C. The beads were washed three times with 1% Triton X-100 in PBS and the eluates were analyzed on SDS–PAGE gels stained with Coomassie Blue to estimate protein concentrations. The sample volumes were adjusted to equalize the amounts of fusion proteins used for protein binding assays in vitro.

Generating hNUDC Deletion Mutant Constructs and In Vitro Translation

The full-length cDNA of hNUDC was originally isolated as a 1.3 kb fragment in a pB42AD vector, designated pB42AD-hNUDC, by a yeast two-hybrid system. Five deletion fragments of hNUDC encoding amino acids 1–132, 1–174, 1–238, 100–238, and 174–331 were amplified by PCR using pB42AD-hNUDC as a template and cloned into the expression vector pET28b (Novagen). In vitro coupled transcription and translation reaction using *E. coli* lysate (Roche) was carried out with the deletion plasmid templates in the presence of ^{35}S-methionine, according to the manufacturer's protocol (Roche).

GST Pull Down Assay

In vitro translated hNUDC peptides or cell extracts of human cell lines were incubated with the GST-Mpl-EC coupled to Glutathione-Sepharose beads in buffer (50 mM HEPES, pH 7.9, 300 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% Nonidet-P40) for 1 h at 4°C. These complexes were washed several times with buffer, and bound proteins were eluted from the resin by boiling the samples in SDS loading buffer. Proteins were separated by 10% SDS–PAGE, followed by autoradiography or Western blot analysis.

Immunoprecipitation and Western blot

Human megakaryocyte cell lines (MEG-01 and Dami) were purchased from Shanghai Cell Research Bank and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells (10 million) obtained from 50 ml of media were homogenized using a Polytron mixer in 1 ml of a detergent lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 2.5% deoxycholic acid, 1% Nonidet P-40, 1 mM EDTA, 1 mM PMSF, and 10% antiprotease cocktail). Cell lysates were clarified by centrifugation at 150,000*g* for 30 min. To 20 μl of the supernatant, 0.5 μg of either polyclonal antibody against MBP-Mpl-EC or monoclonal antibody against hNUDC, and an equal volume of protein A-sepharose beads were added. After incubation at 4°C for 4 h on a rotator, the beads were washed five times with lysis buffer supplemented with 0.02% SDS to reduce nonspecific binding. Bound proteins were eluted with SDS sample buffer and analyzed by Western blot. Proteins were analyzed by 12% SDS–PAGE and transferred to PVDF membranes using a Hoefer SemiPhor semi-dry transfer unit (Amersham Biosciences). The PVDF membrane was blocked with 1 % BSA in Tris-buffered Tween 20 (TBT) overnight at 4°C, followed by incubation with a polyclonal antibody against MBP-Mpl-EC or a monoclonal antibody against hNUDC. Immunoreactive materials were detected by the ECL system, according to the supplier's protocol (Amersham Biosciences).

Immunofluorescence Staining of Human Megakaryocyte Cells

Umbilical cord blood was obtained from Guangdong Hematopoietic Stem Cell Therapy Technology Center. Mononuclear cells were isolated using Ficoll-Hypaque (density, 1.077; Amersham Biosciences) density gradient centrifugation. $CD34^+$ cells were then isolated using the Dynal CD34 Progenitor Cell Selection System (Dynal, Oslo, Norway). Purified $CD34^+$ cells were cultured in serum-free liquid media (Stem SpanTM SFEM, StemCell Technologies, Inc., Vancouver, BC) supplemented with 50 ng/ml TPO. Cells were removed on day 12 and cytospun onto poly-L-lysine coated cover slides.

Cells were fixed in 4% paraformaldehyde in PBS for 45 min, and then permeabilized for 5 min with 0.1% Triton X-100 in PBS. The cells were blocked for at least 1 h at room temperature in PBS containing 10% fetal bovine serum and then washed four times and incubated with the primary antibodies in PBS containing BSA for 1 h at room temperature. After rinsing with PBS five times, DAPI and the secondary antibodies were added.

RESULTS

Identification of a Binding Partner for Mpl in the Yeast Two-Hybrid System

The extracellular domain of Mpl (Mpl-EC) was fused in frame with the bacterial binding domain LexA to form LexA-Mpl-EC, which was used as the bait in a yeast two-hybrid screen for Mpl ligands in a human fetal liver cDNA library. Several positive clones carrying inserts more than 1 kb in length were subjected to DNA sequencing analysis. Surprisingly, all of the inserts were identical to hnudC (GenBank Accession Number BC007280), including a coding region, and 3′ and 5′ untranslated regions. The positive interaction was confirmed by second co-transformation of yeast cells with LexA-Mpl-EC and either pB42AD-hNUDC-F containing 5′- and 3′-untranslated regions or pB42AD-hNUDC containing no 5′- and 3′-untranslated region (Fig. 1A). The empty vector pB42AD showed no positive interaction (Fig. 1A). In addition, a deletion series of hNUDC in pB42AD indicated that amino acids 100–238 were required for interaction with Mpl-EC (Fig. 1B). Constructs corresponding to the N-terminal 1–132, and the C-terminal

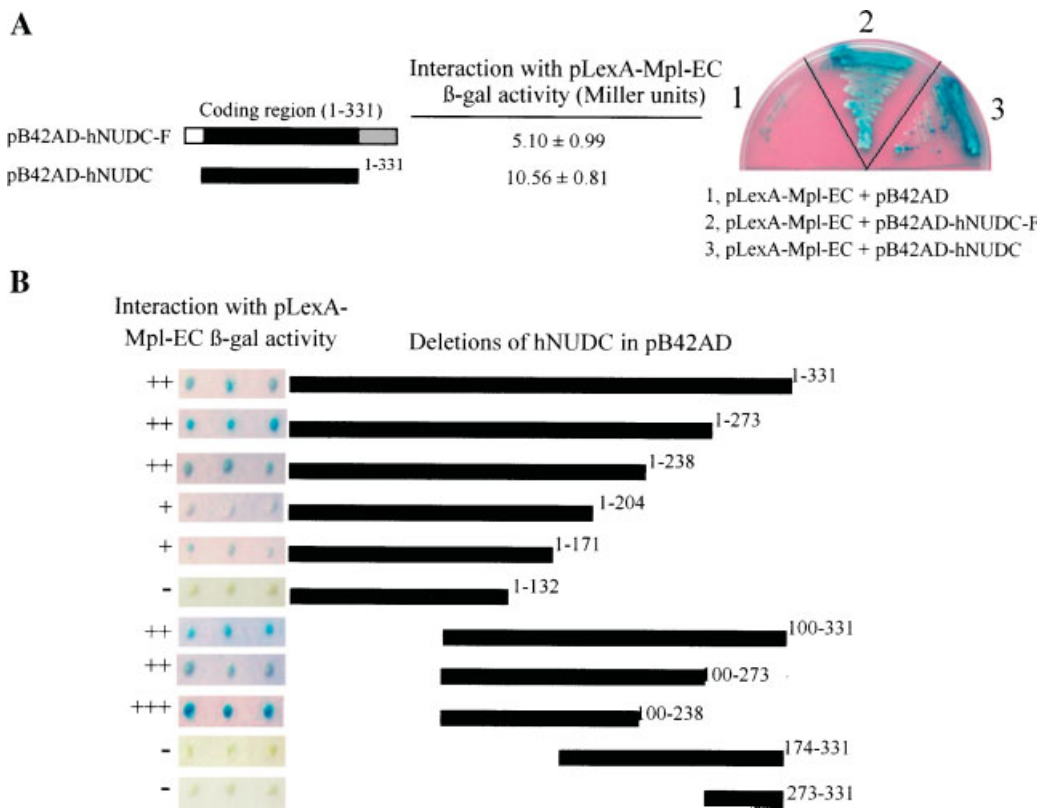

Fig. 1. Determination of the interacting domains of Mpl-EC and hNUDC in a yeast two-hybrid system. **A**: Mpl-EC was fused to LexA. hNUDC-F and hNUDC were fused to B42AD. The criteria for positive interaction were based on their growth on X-gal plates free of leucine (right). β-galactosidase was measured by liquid assay using *O*-nitrophenyl-β-D-galactose as the substrate (left). **B**: The hNUDC deletion mutants (right) and β-galactosidase activity for each construct in the presence of LexA-Mpl-EC in the yeast two-hybrid system (left) are shown. β-Galactosidase activity was determined by colony-lift filter assay. The level of interaction is defined as: +++, very strong; ++ strong; +, weak; −−, undetectable. Measurement of β-galactosidase levels was done in triplicate from three independent colonies in three separate experiments. The numbers reported define amino acid positions.

179–331 and 273–331 amino acids did not interact. Weak interactions with constructs corresponding to amino acids 1–171 and 1–204 (Fig. 1B) indicate that the binding domain of hNUDC resides within its central region from 100–238.

Characterization of the Specificities for the Monoclonal and Polyclonal Antibodies Against hNUDC

Prior to use, monoclonal and polyclonal antibodies raised against rhNUDC were checked for specificity using lysates of cells expressing full-length of rhNUDC (1–331) and its deletion peptides 1–132, 1–174, 1–238, 100-238, and 174–331. The anti-hNUDC monoclonal antibody recognized amino acids 1–174, 1–238, 100–238, and 1–331. These results suggested that the epitope resided within the amino acid residues 100–174 (Fig. 2A). In contrast, the rabbit polyclonal antibody recognized all the recombinant proteins (Fig. 2B).

GST Pull Down Assay

To confirm that amino acids from 100 to 238 of hNUDC are important for binding with Mpl-EC, we used recombinant Mpl-EC and in vitro-translated ^{35}S-hNUDC. Five ^{35}S-labeled deletion constructs and a full-length of hNUDC were incubated with GST-Mpl-EC immobilized on Glutathione-Sepharose beads. In agreement with the two-hybrid results, Mpl-EC strongly associated with the amino acids 1–174, 1–238, and 100–238, no binding of the amino acids of 1–132 and 174–331 to GST-Mpl-EC was evident (Fig. 3A).

To ascertain whether GST-Mpl-EC could also interact with native hNUDC, we performed the GST pull-down experiments with concentrated extracts of human megakaryocyte cells and

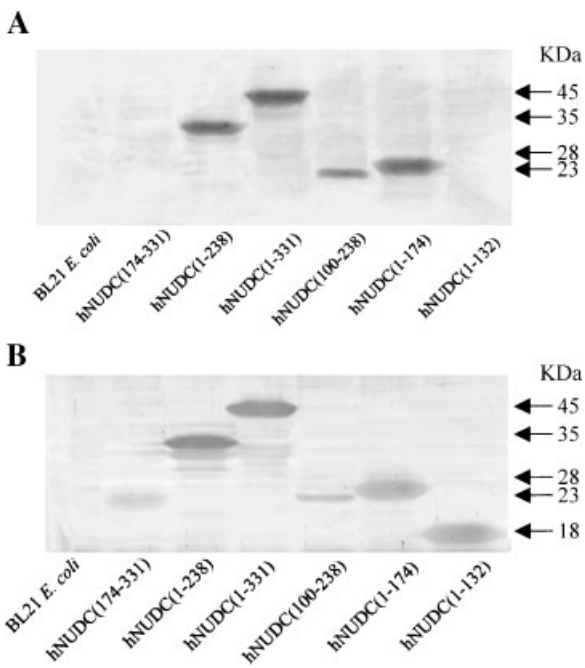

Fig. 2. Western blot of total cell lysates of *E. coli* BL21 harboring a full-length of rhNUDC and its deletion constructs. Cells were cultured at 37°C until the absorbance at 600 nm of the culture reached 0.5–0.6. Expression of proteins was then induced by incubation of the cells for 3 h in the presence of 0.5 mM IPTG. Cells were collected and lysed with SDS loading buffer and samples were separated on a 12% SDS–PAGE gel. **A**: Total cell lysates were analyzed by Western blot using a monoclonal antibody against hNUDC. **B**: Total cell lysates were analyzed by Western blotting using a polyclonal antibody against hNUDC. Cell containing no expression plasmid was used as a negative control.

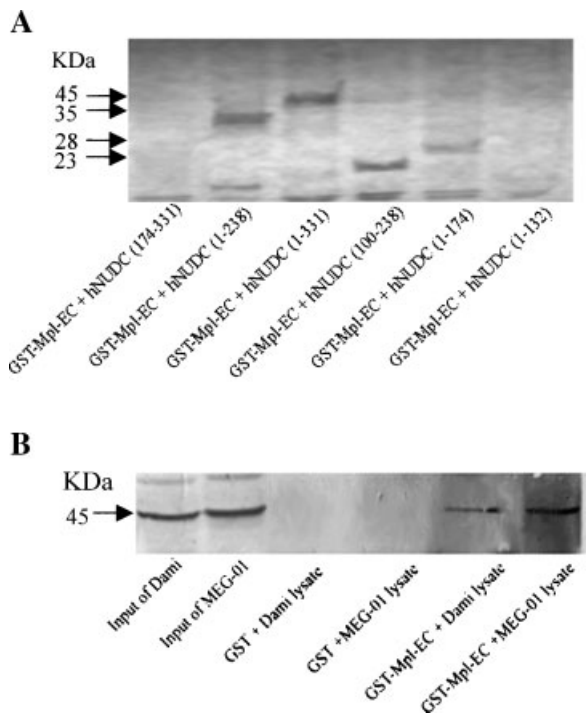

Fig. 3. hNUDC interacts with Mpl-EC in vitro. **A**: GST-Mpl-EC was produced in *E. coli* cells, immobilized on Glutathione-Sepharose beads. Full-length hNUDC and its five deletion derivatives were in vitro ^{35}S-labeled translated and incubated with GST-Mpl-EC bound beads. The bound proteins were pelleted by centrifugation and subsequently washed three times to remove unbound protein. Proteins were eluted and subjected to a 12% polyacrylamide gel, followed by autoradiography. **B**: Immobilized GST-Mpl-EC fusion protein was incubated with MEG-01 and Dami cell extracts, respectively, and the presence of hNUDC in the pull-down complexes was examined by immunoblotting using a monoclonal antibody against hNUDC.

found a strong interaction with native hNUDC (Fig. 3B). No hNUDC from cellular extracts interacted with the Glutathione-Sepharose beads bound with GST only under the same conditions (Fig. 3B).

Co-Immunoprecipitation of hNUDC and Mpl From Human Megakaryocyte Cells

In order to assess whether this interaction exists in mammalian cells, co-immunoprecipitation experiments were performed with the human megakaryocyte cell lines, MEG-01 and Dami, expressing both Mpl and hNUDC as detected by immunoblotting (Fig. 4A,B). Cell extracts were challenged with antibodies directed against either hNUDC or Mpl, and the protein A-sepharose immunopellet from each cell line were analyzed with each antibody to demonstrate the association of hNUDC and Mpl. As shown in Figure 4, immunoprecipitation of Mpl yielded coadsorption of hNUDC (Fig. 4C) and immunoprecipitation of hNUDC yielded coadsorption of Mpl (Fig. 4D).

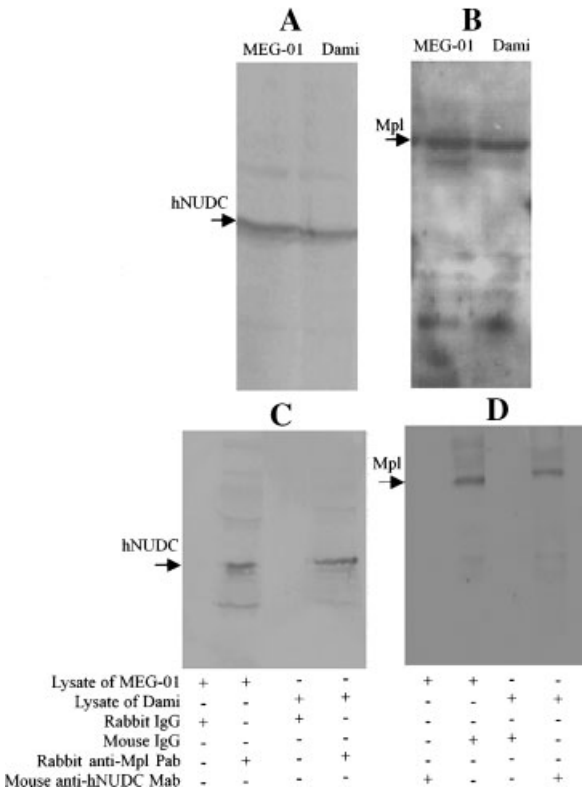

Fig. 4. Co-immunoprecipitation of hNUDC and Mpl in human megakaryocyte cell lines. **A**: Expression of hNUDC in MEG-01 and Dami cells were confirmed by immunoblotting of cell extracts with monoclonal antibody against hNUDC. **B**: Expression of Mpl in MEG-01 and Dami cells were confirmed by immunoblotting of cell extracts with polyclonal antibody against MBP-Mpl-EC. **C**: Cell extracts were immunoprecipitated with polyclonal anti-MBP-Mpl-EC or a rabbit IgG control antibody. hNUDC was visualized by immunoblotting with monoclonal anti-hNUDC. **D**: Cell extracts were immunoprecipitated with monoclonal anti-hNUDC or IgG control antibody. Mpl was visualized by immunoblotting with polyclonal anti-MBP-Mpl-EC.

Detection of Secreted hNUDC in Culture Supernatants of Human Megakaryocyte Lines by Western Blotting

Because our results indicated that hNUDC interacted with the extracellular domain of Mpl, we investigated if hNUDC was a secreted protein. Human megakaryocyte lines MEG-01 and Dami were grown in serum-free medium for 48 h. Culture supernatants were concentrated. Western blots revealed the presence of the hNUDC with a molecular mass of about 45 kDa in concentrated culture media (Fig. 5). Moreover, hNUDC was also found at low levels in mice sera (Fig. 5).

Colocalization of hNUDC and Mpl in Megakaryocyte Cells

Megakaryocytes derived from $CD34^+$ progenitor cells were observed at different stages of maturity for the distribution patterns of hNUDC and Mpl. In immature megakaryocytes, both hNUDC and Mpl were concentrated predominantly along the plasma membrane (Fig. 6A). In maturing megakaryocytes, hNUDC and Mpl appeared to be colocalized in proplatelet forming regions (Fig. 6B). When

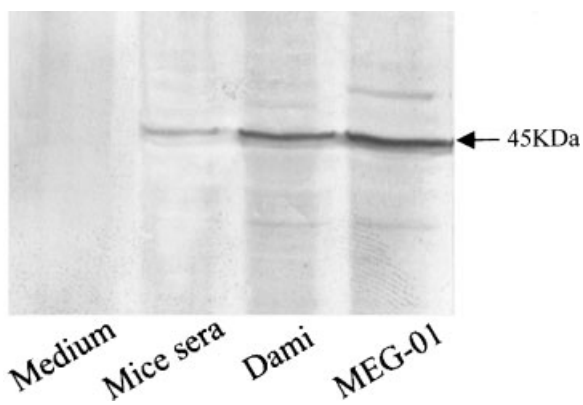

Fig. 5. Detection of secreted hNUDC by Western blotting. MEG-01 and Dami cells were grown in serum-free medium. After removal of cells by centrifugation, the supernatants were equally concentrated fivefold and immunoblotted with monoclonal anti-hNUDC. Concentrated serum-free medium was used as a negative control. hNUDC was also detected in mice sera.

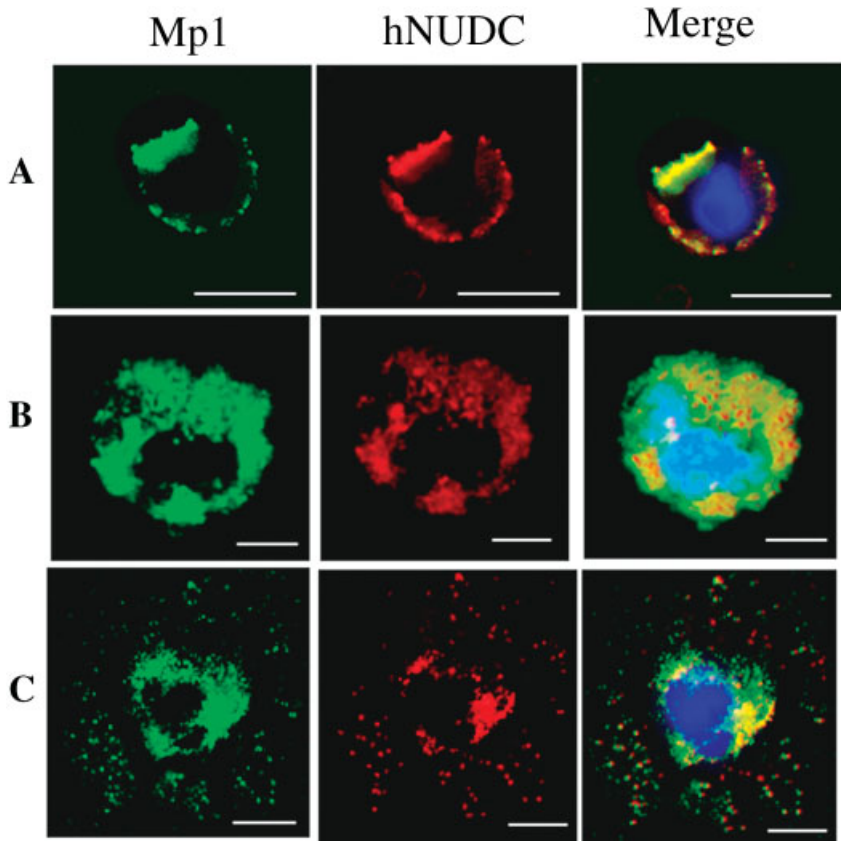

Fig. 6. Colocalization of hNUDC and Mpl in human megakaryocyte cells. Megakaryocytes were generated from $CD34^+$ cells after incubation in serum-free media supplemented with TPO for 12 days. Cells were fixed, permeabilized and co-immunolabeled with monoclonal antibody against hNUDC (red) and polyclonal antibody against MBP-Mpl-EC (green). **A**: hNUDC and Mpl overlap on the plasma membrane of immature megakaryocytes. **B**: hNUDC and Mpl were predominantly colocalized in proplatelet forming region of maturing megakaryocytes. **C**: hNUDC and Mpl overlap in the cytoplasm surrounding the nucleus as well as in proplatelet extensions of maturing megakaryocyte. Colocalizing regions of hNUDC and Mpl appear yellow in merged images. Nuclei were counterstained with DAPI (blue). Bar, 10 μM.

cells formed proplatelets, there was extensive overlap between hNUDC and Mpl (Fig. 6C).

Colocalization of hNUDC and β-Tubulin

Mature megakaryocyte cells were double-immunostained with polyclonal anti-hNUDC and monoclonal anti-β-tubulin. hNUDC and β-tubulin were colocalized around the nucleus and along microtubules of proplatelet extensions (Fig. 7A), whereas small hot spots of hNUDC were observed and in the tips of microtubes (Fig. 7B–E).

DISCUSSION

hNUDC was the only ligand that we isolated from a fetal liver cDNA library in a yeast two-hybrid screen, indicating the specific interaction between hNUDC and Mpl. This interaction was further verified by GST pull-down assays and co-immunoprecipitation of both proteins from human megakaryocyte cells. Additional evidence for an association of hNUDC with Mpl was provided by colocalization of the two proteins in megakaryocyte cells (Fig. 6). Moreover, hNUDC was prominently associated with microtubules around the nucleus and in the proplatelet extensions of megakaryocytes (Fig. 7). Although more extensive studies will be necessary to fully describe these interactions, our studies raise the possibility that the association of hNUDC and Mpl on megakaryocyte developing is of functional significance because addition of hNUDC to megakaryocyte cell cultures induced polyploidization and proplatelet formation more extensively than TPO (data not shown). Furthermore, administration of hNUDC protein substantially enhanced the

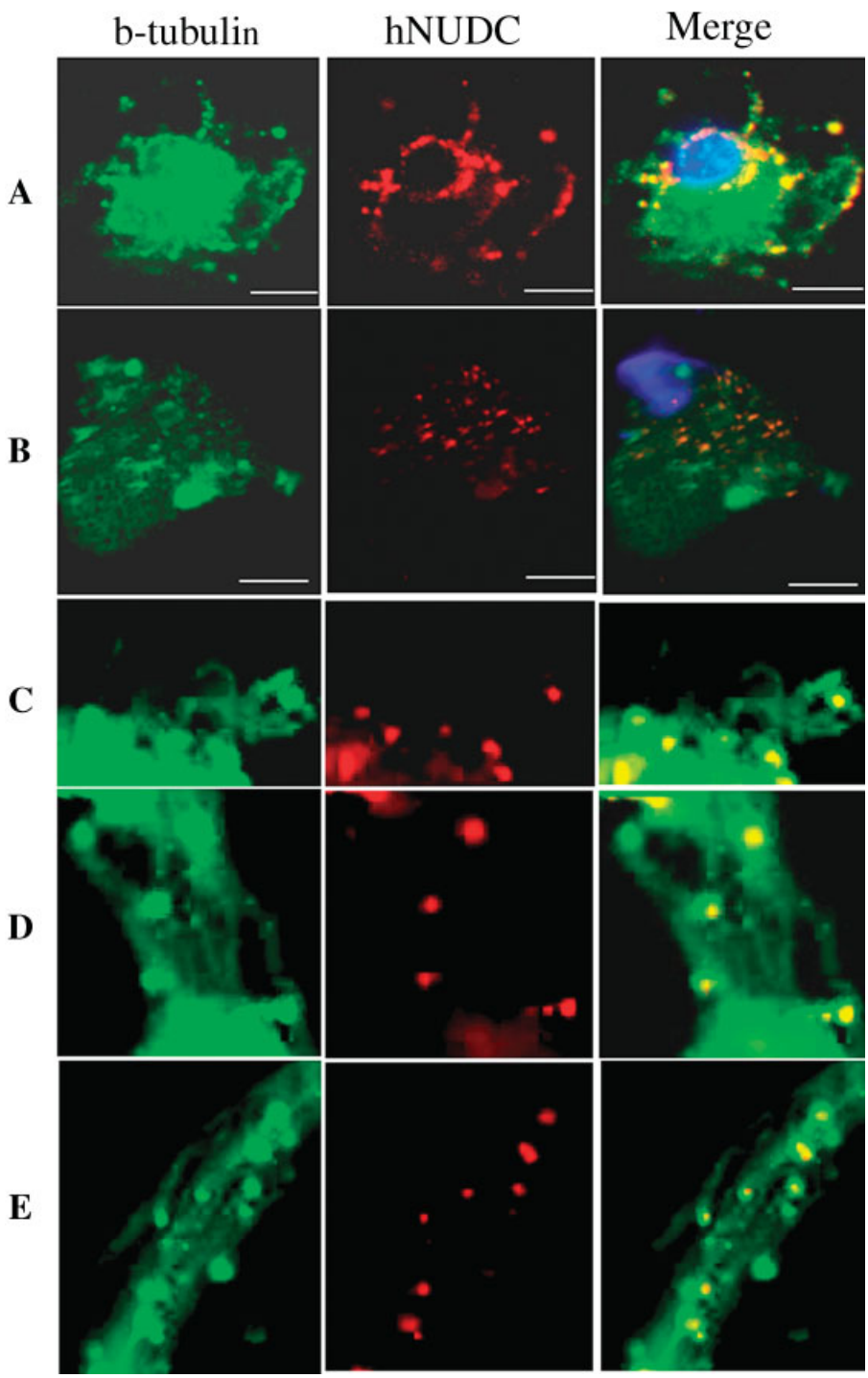

Fig. 7. Colocalization of hNUDC with microtubules in megakaryocytes. Mature megakaryocytes were generated from $CD34^{+}$ cells after incubation in serum-free media supplemented with TPO for 12 days. Cells were fixed, permeabilized and co-immunolabeled with polyclonal antibody against hNUDC (red) and monoclonal antibody against β-tubulin (green). **A**: hNUDC was located around the nucleus and as hot spots along the microtubules of maturing megakaryocyte. **B**: hNUDC was located in the tips of microtubules of maturing megakaryocyte. **C–E**: Detail of proplatelet forming regions showing microtubules and hot-spots of hNUDC. Colocalizing regions of hNUDC and Mpl appear yellow in merged images. Nuclei were counterstained with DAPI (blue). Bar, 10 μM.

number of circulating platelets in normal mice (data not shown).

TPO and hNUDC have no sequence similarity, however, hNUDC binds the same receptor as TPO. hNUDC is a secreted protein in megakaryocyte cells (Fig. 5), indicating that it may act as a cytokine and triggering many of the same responses as TPO. The presence of hNUDC in mice sera (Fig. 5) provided an explanation for the observation of the residual level of platelets in TPO−/− mice. It may be also suggested that under normal conditions hNUDC and TPO synergistically interact to stimulate megakaryocyte maturation because normal in vivo TPO levels are very low (50–150 pg/ml) [Espanol et al., 1999]. However, it is important to note that the observations in this study are most likely not the only means by which hNUDC acts as a cytokine. hNUDC is a nuclear migration factor that forms an intracellular association with Lis1 and the dynein motor complex of microtubules [Aumais et al., 2001]. hNUDC may have secondary effects on the terminal stages of megakaryocyte maturation, including nuclear polyploidization and proplatelets formation, which require microtubule motor proteins [Rothwell and Calvert, 1997; Nagata et al., 1998; Hartwig and Italiano, 2003].

The primary function of hNUDC is considered to be nuclear migration activity because the C-terminus of hNUDC is 53% identical and 73% similar to the full length of fungal NUDC. hNUDC has an extra N-terminal domain (133 aa) compared to the fungal NUDC [Matsumoto and Ledbetter, 1999]. Our deletion analysis in a yeast-two hybrid system indicated that the domain of hNUDC that bound to Mpl spanned a portion of internal amino acids from 100 to 238 (Figs. 1B and 3A). Whereas the C-terminal region of hNUDC was shown to form an intracellular association with Lis1 and the dynein motor complex [Aumais et al., 2001].

hNUDC is a dynein/dynactin—associated nuclear movement protein that plays an important role in the regulation of cellular mitosis and cytokinesis [Aumais et al., 2003]. Several lines of evidence indicate that cytoplasmic dynein/dynactin plays crucial roles in a variety of cellular activities, such as positioning of the mitotic spindle apparatus, movement of mitotic chromosomes, vesicular trafficking, as well as protein sorting events [Goode, 2000; Welte, 2004]. It is intriguing to note that dynein has been reported to localize in human platelets and may be involved in the platelet formation [Rothwell and Calvert, 1997; Hartwig and Italiano, 2003]. The C-terminal region of hNUDC that is homologous to fungal NUDC was shown to form an intracellular association with Lis1/dynein/dynactin motor complex and colocalized with dynein/dynactin at the microtuble-organizing center (MTOC) in neuron and fibroblasts [Aumais et al., 2001]. It has also been shown that ribozyme-mediated downregulation of hNUDC expression levels results in inhibition of proliferation of kidney embryonic cells [Zhang et al., 2002]. Furthermore, in cultured HeLa and C. *elegans* cells, alteration of hNUDC levels resulted in multinucleate cells and the loss of the mitotic kinase, Plk1 from mitotic structures [Aumais et al., 2003]. Since previous studies suggested that the process of polyploidization of megakaryocytes could be due to deregulation of the mitotic spindle late in mitosis, we consider that hNUDC might play a key role in the abortive mitosis. Moreover, because proplatelet extension requires high rates of microtubule-dependent minus-end-directed transport processes [Rothwell and Calvert, 1997; Hartwig and Italiano, 2003], an intriguing possibility emerges that hNUDC may serve as a bridge between Mpl, which is located surface of platelets [Broudy et al., 1997], and dynein, which moves only toward the proplatelet tips along microtubule [Hartwig and Italiano, 2003]. This Mpl/hNUDC/dynein/ complex could be involved in a platelets secretory process through their association with microtubules. The many potential roles of rhNUDC in platelet formation highlight the need for further investigation.

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