FEBS Letters 584 (2010) 3013–3020

FEBS Letters

journal homepage: www.FEBSLetters.org

The nucleoporin Nup153 maintains nuclear envelope architecture and is required for cell migration in tumor cells

Lixin Zhou, Nelly Panté *

Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC, Canada V6T 1Z4

ARTICLE INFO

Article history:
Received 25 March 2010
Revised 12 May 2010
Accepted 13 May 2010
Available online 24 May 2010

Edited by Ulrike Kutay

Keywords:
Nucleoporin
Nucleoporin 153
Nuclear pore complex
Cell migration
Nuclear lamina
Cytoskeleton

ABSTRACT

Nucleoporin 153 (Nup153), a component of the nuclear pore complex (NPC), has been implicated in the interaction of the NPC with the nuclear lamina. Here we show that depletion of Nup153 by RNAi results in alteration of the organization of the nuclear lamina and the nuclear lamin-binding protein Sun1. More striking, Nup153 depletion induces a dramatic cytoskeletal rearrangement that impairs cell migration in human breast carcinoma cells. Our results point to a very prominent role of Nup153 in connection to cell motility that could be exploited in order to develop novel anti-cancer therapy.

Structured summary:
MINT-7893777: *Lamin-A/C* (uniprotkb:P02545) and *NUP153* (uniprotkb:P49790) *colocalize* (MI:0403) by *fluorescence microscopy* (MI:0416)
MINT-7893761: *sun1* (uniprotkb:Q9D666) and *Lamin-A/C* (uniprotkb:P02545) *colocalize* (MI:0403) by *fluorescence microscopy* (MI:0416)

1. Introduction

The mammalian nuclear pore complex (NPC) is composed of multiple copies of 30 different nucleoporins [1]. The nucleoporin 153 (Nup153) is strategically positioned at the NPC nuclear basket to interact with the nuclear lamina, and at least in vitro, it interacts with lamin B_3 [2]. Recent findings demonstrate that the nuclear lamina is connected with cytoskeletal elements through the linker of nucleoskeleton and cytoskeleton (LINC) complex that spans the double membrane of the nuclear envelope (NE) [3,4]. This complex is formed by outer nuclear membrane Nesprins (actin-binding proteins) and inner nuclear membrane Sun proteins (lamin-binding proteins), which are tethered together at the perinuclear space. To determine whether Nup153 plays a role in the organization of the NE and thereby indirectly impacts the cytoskeletal architecture and mechanical properties of the cell, we employed an siRNA approach to reduce the cellular expression of Nup153. Our data suggest that Nup153 plays an essential role in maintaining nucleoskeleton and cytoskeleton architecture, and is required for cell cycle progression and cell migration.

2. Materials and methods

2.1. Cells, antibodies and Nup153 RNAi

Cells were cultured at 37 °C and 5% CO_2 in complete Dulbecco's modified Eagle medium supplemented with 10% FBS, penicillin–streptomycin, and 2 mM L-glutamine. Cells used were: HeLa cells (American Type Culture Collection), tetracycline-inducible HeLa cells stably expressing Sun1 carrying a C-terminal GFP tag (Sun1-GFP [5]; courtesy of Dr. B. Burke and Dr. K. Roux, University of Florida), human breast carcinoma MDA231 cells and human fibrosarcoma HT1080 cells (courtesy of Dr. C. Roskelley, University of British Columbia).

The following antibodies were used: monoclonal SA1 against human Nup153 [6] (provided by Dr. B. Burke, Institute of Molecular and Cell Biology, Singapore), polyclonal anti-lamin A/C antibody (sc-20681; Santa Cruz Biotechnology), monoclonal anti-actin (AC-40; Sigma), monoclonal anti-α-tubulin (Sigma), polyclonal anti-γ-tubulin (Sigma), monoclonal anti-fibrillarin (38F; Abcam),

Abbreviations: cNLS, classical nuclear localization sequence; EdU, ethynyl deoxyuridine; LINC, linker of nucleoskeleton and cytoskeleton; MTOC, microtubule-organizing centre; NE, nuclear envelope; NPC, nuclear pore complex; Nup153, nucleoporin 153

* Corresponding author. Fax: +1 604 822 2416.
E-mail address: pante@zoology.ubc.ca (N. Panté).

0014-5793/$36.00
doi:10.1016/j.febslet.2010.05.038

and monoclonal Golgi (GM130; BD Transduction Laboratories). Phalloidin-FITC was obtained from Sigma. For indirect immunofluorescence analysis, secondary antibodies were from Invitrogen. For Western blot, the isotype-specific HRP-coupled secondary antibodies were from Amersham Pharmacia.

For RNAi, cells were either mock transfected or transfected with siRNA (Dharmacon) against Nup153 at a final concentration of 10 nM using lipofectamine RNAiMAX (Invitrogen) according to the manufacture's instructions. The sequence used corresponded to nucleotide 2593–2615 of human Nup153 (AAGGCAGACUCUACCAAAUGUTT). Expression of Nup153 was assessed by Western blot and immunofluorescence microscopy one, two and three days after transfection.

2.2. Immunoblotting and immunofluorescence microscopy

Whole cells extracts were prepared by cell lysis in RIPA buffer (150 mM NaCl, 50 mM Tris–HCl pH 8.0, 0.5 mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS, 0.5% NP-40, 10 mM PMSF, 1 μm pepstatin, 10 μg/ml aprotinin, and 2 mg/ml leupeptin). The lysates were incubated for 20 min on ice. After centrifugation at 15 000×*g* for 10 min at 4 °C, the supernatants were mixed with SDS–PAGE sample buffer. Protein concentration was determined using the BCA™ protein assay kit (Pierce, Rockford, IL), and aliquots with equal amounts of proteins were loaded on SDS–PAGE. Proteins were transferred to nitrocellulose membranes (Bio-Rad), and specific proteins were detected with indicated primary antibodies and

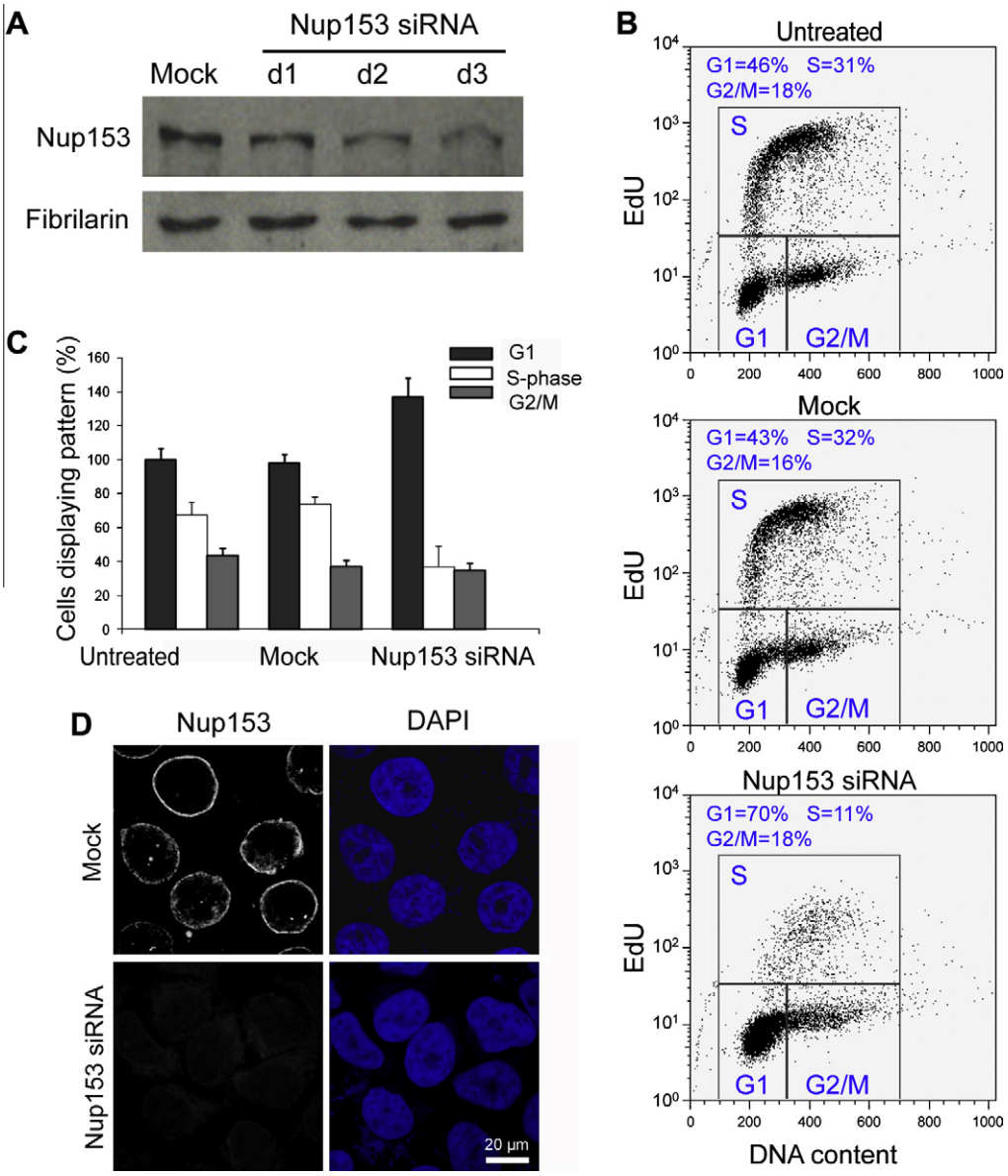

Fig. 1. Nup153 depletion by RNAi alters the cell cycle. (A) Western blot of extracts from mock-transfected and Nup153-siRNA-transfected HeLa cells prepared 1, 2 and 3 days after transfection, and probed with the SA1 antibody to detect Nup153, as well as an anti-fibrillarin antibody as an internal standard. (B) Representative flow cytometric profiles of HeLa cells untransfected, mock transfected and Nup153-siRNA transfected, as measured after a 30 min incorporation of EdU. The dot histograms show the fluorescent intensity of the staining for the incorporated EdU (y axis) and the fluorescent DNA probe 7-amino-actinomycin D (7-AAD; x axis). The percentage of cells in the G1, G2/M and S phases of the cell cycle are indicated in the upper left corner of the plots. (C) Percentage of cells in each cell cycle phase, presented as a percentage of the G1 phase of untransfected cells. Shown are the mean values and standard error measured for three independent experiments. (D) Immunofluorescence microscopy of Nup153-siRNA-treated HeLa cells reveals a loss of NE-associated Nup153, 48 h post-transfection. Cells were probed with an anti-Nup153 antibody, and DNA was detected by staining with DAPI.