Biochemical and Biophysical Research Communications 434 (2013) 155–161

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Nucleoporin Nup98 mediates galectin-3 nuclear-cytoplasmic trafficking

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ARTICLE INFO

Article history:
Received 4 March 2013
Available online 26 March 2013

Keywords:
Nucleoporin
Nuclear pore complex
Galectin-3
Nuclear transport
Cancer progression

ABSTRACT

Nucleoporin Nup98 is a component of the nuclear pore complex, and is important in transport across the nuclear pore. Many studies implicate nucleoporin in cancer progression, but no direct mechanistic studies of its effect in cancer have been reported. We show here that Nup98 specifically regulates nucleus–cytoplasm transport of galectin-3, which is a ß-galactoside-binding protein that affects adhesion, migration, and cancer progression, and controls cell growth through the ß-catenin signaling pathway in cancer cells. Nup98 interacted with galectin-3 on the nuclear membrane, and promoted galectin-3 cytoplasmic translocation whereas other nucleoporins did not show these functions. Inversely, silencing of Nup98 expression by siRNA technique localized galectin-3 to the nucleus and retarded cell growth, which was rescued by Nup98 transfection. In addition, Nup98 RNA interference significantly suppressed downstream mRNA expression in the ß-catenin pathway, such as cyclin D1 and FRA-1, while nuclear galectin-3 binds to ß-catenin to inhibit transcriptional activity. Reduced expression of ß-catenin target genes is consistent with the Nup98 reduction and the galectin-3–nucleus translocation rate. Overall, the results show Nup98's involvement in nuclear–cytoplasm translocation of galectin-3 and ß-catenin signaling pathway in regulating cell proliferation, and the results depicted here suggest a novel therapeutic target/modality for cancers.

1. Introduction

Cancer development and progression is a complex process that involves functional and genetic abnormalities, which lead to altered gene expression and overall cell function. Recently some nucleoporins have been linked to cancers [1,2]. Nucleoporins are the main components of the nuclear pore complex (NPC), which bridge the nuclear envelope to form a transport channel between the nucleus and the cytoplasm [3]. Several cellular processes, including molecular nucleocytoplasmic transport, are controlled by nucleoporins. Interestingly, several nucleoporins were linked to cancer; Nup88 was found to be overexpressed in ovarian, breast, colorectal cancer, etc. [4]. Rae1 expression was abberant in breast cancer [5], and Nup98 and translocated promoter region (Tpr) were observed as chimeric fusion proteins by chromosomal translocations in leukemia, gastric, and thyroid cancer [6,7]. Although nucleoporins have been the objects of much investigation, very little is known of their function in cancer progression.

A key factor in cancer progression is galectin-3—a member of an evolutionary conserved family of ß-galactoside-binding proteins, which is ubiquitously expressed and involved in diverse biological functions [8]. Galectin-3 plays several significant roles in cancer progression, involving cell growth, adhesion, migration, invasion, angiogenesis and apoptosis [9]. Interestingly, galectin-3 shuttles between the cytoplasm and the nucleus and its nuclear import involves karyopherins (importins) [10]. Galectin-3's localization in two cellular compartments is associated with cancer progression; reportedly, cytoplasmic galectin-3 promotes tumor progression, whereas nuclear galectin-3 suppresses malignancy [11]. Galectin-3 expression in the nucleus is greatly decreased in colon and prostate cancer; marked reduction of nuclear galectin-3 was seen in tongue cancer progression [12,13]. Cytoplasmic galectin-3 expression translocated from the nucleus also exhibits anti-apoptotic activity [14]. Galectin-3 localization in the nucleus or cytoplasm is critical for cancer progression, although the mechanism of translocation is yet to be determined.

Abbreviations: APC, adenomatous polyposis coli; CK1, casein kinase 1; NPC, nuclear pore complex; Nup, nucleoporin; RNAi, RNA interference; siRNA, small interfering RNA; Tpr, translocated promoter region.

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0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.03.052

Karyopherins mediate nuclear protein transport in association with nuclear pore complex proteins—mainly, the nucleoporin Nup98 [15]. Thus, here we examined the mechanism of galectin-3 translocation regulation by Nup98 and established critical roles for Nup98 and galectin-3 in cancer progression.

2. Materials and methods

2.1. Reagents and antibodies

Anti-Nup98 antibody was kindly donated from Dr. Günter Blobel (Rockefeller University). Anti-galectin-3 antibody was described elsewhere [14]. Anti-α-tubulin and Nup62 antibodies were obtained from Sigma (St. Louis, MO). Anti-Nup88 and ß-catenin antibodies were from Transduction Laboratories (Lexington, KY). Anti-Nup358 and CRM1 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Nup214 and Nup153 antibodies were from Abcam (Cambridge, MA). Anti-GFP antibody was from Invitrogen (Carlsbad, CA). Secondary antibodies were from Molecular Probes (Eugene, OR). Casein kinase 1 (CK1) inhibitor D4476 was purchased from Cayman Chemical Co. (Ann Arbor, MI).

2.2. Cell culture

Human cervical cancer cell line HeLa and human breast cancer cell line MCF7 were obtained from the American Type Culture Collection (Manassas, VA). Human breast cancer cell lines BT-549 and MDA-MB-231 were kindly provided by Dr. Erik W. Thompson (University of Melbourne). The establishment of stable BT-549-galectin-3 clones was described previously [14]. All cells were cultured in Dulbecco's modified Eagle's medium with 10% heat-inactivated fetal bovine serum.

2.3. Transfections

The plasmid encoding full-length human Nup98 tagged with GFP was described previously [16,17]. The $Nup98_{1-505}$ domain (Nup98-N) and the $Nup98_{506-920}$ domain (Nup98-C) were subcloned by PCR into the pEGFP-C1 vector (Clontech Laboratories, Mountain View, CA). The full-length Nup62 coding region was PCR-amplified from cDNA and subcloned into pEGFP-C1 vector. All constructs were confirmed by DNA sequencing. All cloning procedures were essentially carried out as described previously [17]. Cells were transfected with plasmids using Lipofectamine 2000 (Invitrogen). siRNA duplexes targeting Nup98 (sc-43535), Nup62 (sc-36107), and control siRNA (sc-37007) were purchased from Santa Cruz Biotechnology. siRNA transfections were performed using Lipofectamine 2000.

2.4. Immunoblotting

Equal amounts of the proteins were separated on SDS–PAGE gels and transferred to 0.2 μm polyvinylidene fluoride membrane at 15 V, 30 mA overnight at 4 °C. The membrane was blocked with 5% skim milk solution in phosphate-buffered saline (PBS) for 1 h at room temperature, and then incubated with primary antibody and secondary antibodies conjugated with horseradish peroxidase. Blots were visualized by enhanced chemiluminescence system.

2.5. Immunoprecipitation

Cell lysates containing equal amounts of protein were precleared and then mixed together with protein A/G bead slurry (Santa Cruz Biotechnology) adding various antibodies as specified, and incubated for 1 h at 4 °C with rocking. The beads were then washed five times with lysis buffer. After extensive washing with lysis buffer, proteins were eluted with SDS–PAGE blue loading buffer, followed by boiling, and subjected to immunoblotting.

2.6. Reverse transcription-PCR (RT-PCR)

Total RNA was extracted using TRIzol Reagent (Invitrogen). The cDNA for PCR template was generated by using ThermoScript™ RT-PCR System for First-Strand cDNA Synthesis (Invitrogen). Each PCR cycle consisted of: 1 min at 95 °C, 1 min at 55 °C and 2 min at 72 °C for cyclin D1, FRA-1 and GAPDH. PCR-amplified products were electrophoresed in 1.5% agarose gel and stained with ethidium bromide. The primer sets were as follows: for cyclin D1, forward: 5′-AACTACCTGGACCGCTTCCT-3′ and reverse: 5′-CCACTTGAGCTTGTTCACCA-3′; for FRA-1, forward: 5′-AGCTGCAGAAGCAGAAGGAG-3′ and reverse: 5′-GGAGTTAGGGAGGGTGTGGT-3′; for GAPDH, forward: 5′-GAGTCAACGGATTTGGTCGT-3′ and reverse: 5′-TTGATTTTGGAGGGATCTCG-3′. Each expression was standardized using GAPDH as an internal control. Density of each band was quantitated with ImageJ software.

2.7. Immunofluorescence

Cells seeded on coverslips were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature. The fixed cells were washed thrice with PBS and then permeabilized with 0.2% Triton X-100 in PBS for 15 min. The cells were blocked with 4.0% BSA in PBS for 30 min and then labeled with primary antibody for overnight at 4 °C. After incubation, the cells were washed with 0.05% Triton X-100 in PBS and incubated with secondary antibodies conjugated with fluorophores for 1 h at room temperature in the dark. After washing with PBS, samples were mounted onto coverslips with Pro-Long Gold Antifade reagent (Invitrogen) and were examined on a Zeiss LSM5 EXCITER confocal microscope, and all images were acquired using an aplan-Apochromat 63× or 100× with a 1.4-N.A. objective.

2.8. Cell growth

Cell growth was determined by Trypan blue dye exclusion assay. Cells were plated into 12-well culture dishes. After incubation, the cells were harvested by trypsinization, centrifuged, and cell pellets were then resuspended in PBS. Cells were stained with trypan blue, and counted using a hematocytometer.

2.9. Statistical analysis

Data are expressed as means ± SD for triplicate determinations. Comparisons between groups was determined using unpaired Student's t tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Nup98 interacts with galectin-3

We have reported that galectin-3 is transported into the nucleus by karyopherins, and it has also been suggested that karyopherins bind to some nucleoporins, including Nup98 [10,15]. These findings prompted us to investigate a possible link between nucleoporins and galectin-3. To examine whether galectin-3 associated with nucleoporins in HeLa cells, we used immunoprecipitation assays to detect coprecipitating Nup98, along with galectin-3; conversely, we immunoprecipitated Nup98 using anti-galectin-3 antibodies (Fig. 1A). Consistent with the immunoprecipitation data, we found that Nup98 colocalized with galectin-3 on the